

Plenary Session 1

Induced Mutations in Food and Agriculture

The Role of Induced Mutations in World Food Security

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Abstract

Physical availability and economic accessibility of food are the most important criteria of food security. Induced mutations have played a great role in increasing world food security, since new food crop varieties embedded with various induced mutations have contributed to the significant increase of crop production at locations people could directly access. In this paper, the worldwide use of new varieties, derived directly or indirectly from induced mutants, was reviewed. Some highlights are: rice in China, Thailand, Vietnam, and the USA; barley in European countries and Peru, durum wheat in Bulgaria and Italy, wheat in China, soybean in China and Vietnam, as well as other food legumes in India and Pakistan. An exact estimate of the area covered by commercially released mutant cultivars in a large number of countries is not readily available, but the limited information gathered clearly indicates that they have played a very significant role in solving food and nutritional security problems in many countries.

Introduction

Ever since the epoch-making discoveries made by Muller [1] and Stadler [2] eighty years ago, a large amount of genetic variability has been induced by various mutagens and contributed to modern plant breeding. The use of induced mutations over the past five decades has played a major role in the development of superior plant varieties all over the world (Fig. 1a). Among the mutant varieties, the majority are food crops (Fig. 1b).

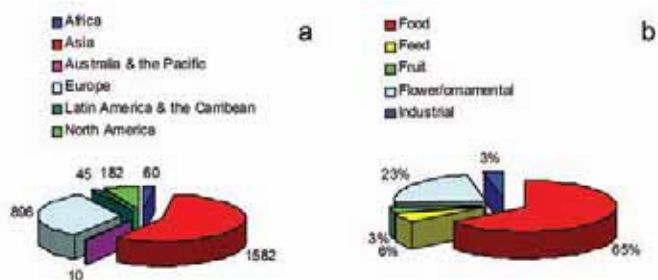


Figure 1 Plant varieties derived from induced mutants. a) The number of mutant varieties in different continents and b) proportion of various plant types Source: FAO/IAEA Database of mutant varieties and genetic stock, <http://mvgs.iaea.org>, 17 November, 2008.

Food security has been variously defined in economic jargon, but the most widely accepted definition is the one by the World Bank [3] – “access by all people at all times to enough food for an active, healthy life”. Likewise, the World Food Summit at Rome in 1996 also known as Rome

Declaration on World Food Security [4] on food plan action observed that, “Food security at the individual, household, national and global level exists where all people at all times have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life”. In both definitions, emphasis has been given to physical availability and economic accessibility of food to the people. The mutant varieties are often grown by farmers in their fields, and any increase of food production resulted from the cultivation of the mutant varieties could be translated into increased food security, since this should be accessible for the people in need.

A detailed review on the global impact of mutation-derived varieties developed and released in major crops all over the world has been published by Ahloowalia, *et al.* [5]. Several papers presented in this Symposium have also elaborated the contribution of induced mutations to food security in either a particular country or a particular crop. Herewith, we present the overall role of induced mutations worldwide, by continent and country, with emphasis on those countries not already discussed in papers which are included in this book.

ASIA

According to the FAO/IAEA database [6], more than half of the mutant derived varieties were developed in Asia (Fig. 1); China, India, and Japan are the three countries that released the largest number of mutant varieties in the world. Some important achievements are summarized here.

China

In China, the mutant rice variety ‘Zhefu 802’ deriving from var. ‘Simei No. 2’, induced by Gamma-rays, has a short growing period (105 to 108 days), high yield potential even under poor management and infertile conditions, wide adaptability, high resistance to rice blast, and tolerance to cold [5]. Therefore, it was the most extensively planted conventional rice variety between 1986 and 1994. Its cumulative planted area reached 10.6 million ha during that period [7]. Two other mutant rice varieties, Yuanfengzao (1970’s) and Yangdao # 6 (2000’s), developed and released before and after Zhefu 802, are further mutant varieties that had been grown on annual scales up to one million ha (Ministry of Agriculture, China, unpublished data). Using a pollen irradiation technique, two new high-quality, high-yield, and early maturity mutant varieties – Jiahezazhan and Jiafuzhan, resistant to blast and plant-hopper, as well as endowed with a wide adaptability - were developed and are now planted annually on 363,000 ha in Fujian province of China [8].

China has also been successful in breeding soybean varieties using mutation techniques. For example, the mutant soybean varieties developed by the Genetics Institute of the Chinese Academy of Sciences possess different excellent traits such as high yield, good grain quality, disease/insect resistance, or drought/salt tolerance. The total area planted with these varieties was more than 1×10^7 ha. [9]. The “Henong series” soybean mutant cultivars, developed and released by the Soybean Institute of Heilongjiang Academy of Agricultural Sciences, as well

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as another variety, Tiefeng18, were grown on an area of more than 2.33×10^6 ha and 4×10^6 ha respectively (Ministry of Agriculture, China, unpublished data).

China has developed and released a large number of high yielding groundnut mutant varieties during the last few decades. The cumulative cultivated area of the more than 35 mutant cultivars released accounts for about 20% of the total area under groundnut in China [10].

Table 1. Number of released mutant varieties in 57 crop species in India

SN	Latin name	Common name	No. of varieties
1	<i>Ablemoschus esculentus</i> L. Moench	Okra	2
2	<i>Arachis hypogaea</i> L.	Groundnut	18
3	<i>Bougainvillea spectabilis</i> Wild	Bougainvillea	13
4	<i>Brassica juncea</i> L.	Mustard	9
5	<i>Cajanus cajan</i> L. Millsp.	Pigeonpea	5
6	<i>Capsicum annuum</i> L.	Green pepper	1
7	<i>Carica papaya</i> L.	Papaya	1
8	<i>Chrysanthemum</i> sp.	Chrysanthemum	49
9	<i>Cicer arietinum</i> L.	Chickpea	8
10	<i>Corchorus capsularis</i> L.	White jute	2
11	<i>Corchorus olitorius</i> L.	Tossa jute	3
12	<i>Curcuma domestica</i> Val.	Turmeric	2
13	<i>Cymbopogon winterianus</i> Jowitt.	Citronella	9
14	<i>Cyamopsis tetragonoloba</i> L.	Cluster bean	1
15	<i>Dahlia</i> sp.	Dahlia	11
16	<i>Dolichos lablab</i> L.	Hyacinth bean	2
17	<i>Eleusine coracana</i> L.	Finger millet	7
18	<i>Gladiolus</i> L.	Gladiolus	2
19	<i>Glycine max</i> L. Merr.	Soybean	7
20	<i>Gossypium arborium</i> L.	Desi cotton	1
21	<i>Gossypium hirsutum</i> L.	American cotton	8
22	<i>Helianthus annuus</i> L.	Sunflower	1
23	<i>Hibiscus sinensis</i> L.	Hibiscus	2
24	<i>Hordeum vulgare</i> L.	Barley	13
25	<i>Hyocymus niger</i>	Indian henbane	1
26	<i>Lantana depressa</i> L.	Wild sage	3
27	<i>Lens culinaris</i> L. Medik.	Lentil	3
28	<i>Luffa acutangula</i> Roxb.	Ridged gourd	1
29	<i>Lycopersicon esculentum</i> M.	Tomato	4
30	<i>Matricaria cammomilla</i>	German chamomile	1
31	<i>Mentha spicata</i>	Spearmint	1
32	<i>Momordica charantia</i> L.	Bitter gourd	1
33	<i>Morus alba</i> L.	Mulberry	1
34	<i>Nicotiana tabacum</i> L.	Tobacco	1
35	<i>Oryza sativa</i> L.	Rice	42
36	<i>Papaver somaniferum</i> L.	Opium poppy	2
37	<i>Pennisetium typhoides</i> L.	Pearl millet	5
38	<i>Phaseolus vulgaris</i> L.	French bean	1
39	<i>Pisum sativum</i> L.	Pea	1
40	<i>Plantago ovata</i> L.	Isabgol	2
41	<i>Polyanthus tuberosa</i> L.	Tuberose	2
42	<i>Portulaca grandiflora</i> L.	Portulaca	11
43	<i>Ricinus communis</i> L.	Castor	4
44	<i>Rosa</i> sp.	Rose	16
45	<i>Sachharum officinarum</i> L.	Sugarcane	9
46	<i>Sesamum indicum</i> L.	Sesame	5
47	<i>Setaria italica</i> L.	Foxtail millet	1
48	<i>Solanum khasianum</i> Clarke	Khasianum	1
49	<i>Solanum melongena</i> L.	Brinjal	1
50	<i>Solenostemon rotundifolius</i>	Coleus	1
51	<i>Trichosanthes anguina</i> L.	Snake gourd	1
52	<i>Trifolium alexandrinum</i> L.	Egyptian clover	1
53	<i>Triticum aestivum</i> L.	Wheat	4
54	<i>Vigna aconitifolia</i> Jacq. M.	Moth bean	5
55	<i>Vigna mungo</i> L. Hepper	Blackgram	9
56	<i>Vigna radiata</i> L. Wiczeck	Mungbean	15
57	<i>Vigna unguiculata</i> L. Walp.	Cowpea	10
		Total	343

India

In India, sustained efforts for crop improvement through induced mutations were initiated during the second half of the 1950s, although the world's very first mutant variety of cotton, MA-9 induced by X-rays, endowed with drought tolerance, was released in 1948 by India [11]. The Indian Agricultural Research Institute (IARI) in New Delhi; Bhabha Atomic Research Center (BARC) in Mumbai, Tamil Nadu Agricultural University (TNAU) in Coimbatore, and the National Botanical Research Institute (NBRI) in Lucknow, are some of the major research centers actively engaged in mutation breeding for several crops and have contributed substantially to the development and release of a large number of mutant varieties. Kharkwal, *et al.* [11] in 2004 listed a total of 309 mutant cultivars of crops, belonging to 56 plant species that were approved and/or released in India by the end of the twentieth century. An updated list of 343 mutant cultivars released in India is given in **Table 1**. The largest number of mutant cultivars have been produced in ornamentals (119), followed closely by legumes (85) and cereals (74).

The mutant cultivars have contributed immensely in augmenting the efforts of Indian plant breeders in achieving the target of food self-sufficiency and strong economic growth. Mutation breeding has thus significantly contributed to the increased production of rice, groundnut, chickpea, mungbean, urdbean, and castor in the Indian sub-continent. While authentic information on the area covered under these cultivars is unfortunately lacking in general, some data is available as summarized below.

The mungbean varieties Co-4, Pant Mung-2, and TAP-7, though released in the early 1980s, are still being grown widely around the country. The variety TARM-1, resistant to powdery mildew and YMV diseases, is the first of its kind to be released for *rabi*/rice fallow cultivation. Four of the nine mutant varieties of blackgram (urdbean) released in India have been developed at the Bhabha Atomic Research Centre (BARC) in Mumbai. One of these mutant varieties, TAU-1, has become the most popular variety in Maharashtra State, occupying an area of about 500,000 hectares (over 95% of the total area under urdbean cultivation in Maharashtra). Since 1990, the Maharashtra State Seed Corporation, Akola, has distributed about 200,000 quintals of certified seeds of TAU-1 to the farmers, which has resulted in an additional production of about 129,000 quintals of urdbean annually in Maharashtra. The notional income generated by additional production amounts to Rs. 300 crores (about 60 million US dollars) annually [11].

Several high-yielding rice mutants were released under the 'PNR' series; some of these were also early in maturity and had short height [12]. Among these, two early ripening and aromatic mutation-derived rice varieties, 'PNR-381' and 'PNR-102', were very popular with farmers in Haryana and Uttar Pradesh States. No data is available on the actual area planted with these varieties. However, based on the rate of fresh seed replacement by farmers and the distribution of breeder seed, foundation seed, and certified seed, as well as on the basis of data obtained from IARI, the value of rice (paddy) production would be 1,748 million US dollars per year [5].

Chickpea: The four high yielding and Ascochyta blight and wilt disease resistant chickpea mutant varieties Pusa – 408 (Ajay), Pusa – 413 (Atul), Pusa – 417 (Girnar), and Pusa – 547, developed at I.A.R.I., New Delhi, and released by the Indian government for commercial cultivation, are the first examples of direct use of induced micro-mutants in a legume crop in the world. Beside high yield performance under late sown crop, chickpea mutant variety Pusa – 547, released in 2006 for farmers' cultivation, has attractive bold seeds, thin testa, and good cooking quality [11, 13, 14, 15].

The success of mutant varieties released is also evident from the large quantities of breeder seed of several mutant varieties at the national level (**Table 2**).

The release of 'TG' (Trombay groundnut) cultivars of groundnut in India has contributed millions of dollars to the Indian economy. Detailed information on the great success of mutation breeding of groundnut and legumes, as well as their contribution to food security in India, can be found in another paper in this book [16].

Table 2. Breeder seed (BS) production of mutant varieties in India (2003–2008)

S.No.	Crop	Mutant variety	BS (kg)	Period
1	Groundnut	TAG-24	427,500	5 yr
2	Groundnut	TG-26	78,600	5 yr
3	Groundnut	TPG-41	37,100	2 yr
4	Barley	RD-2035	53,600	4 yr
5	Soybean	NRC-7	50,200	5 yr
6	Chickpea	Pusa-547	9,100	1 yr

Japan

More than 200 direct-use mutant varieties generated through gamma irradiation, chemical mutagenesis, and somaclonal variations, have been registered in Japan [17]. About 61% of these were developed through mutation induction by Gamma-ray irradiation at the Institute of Radiation Breeding. In 2005, two direct-use cultivars and 97 indirect-use cultivars made up for approximately 12.4% of the total cultivated area in Japan. More information about mutant varieties and their contribution to food production in Japan is available in Nakagawa's paper in this book [17].

Thailand

The contribution of induced mutation to food production in Thailand is best reflected by the work on rice. Two aromatic *indica* type varieties of rice, 'RD6' and 'RD15', released in 1977 and 1978 respectively, were derived from gamma irradiated progeny of the popular rice variety 'Khao Dawk Mali 105' ('KDML 105'). RD6 has glutinous endosperm and retained all other grain traits, including the aroma of the parent variety. RD15, on the other hand, is non-glutinous and aromatic like the parent, but ripens 10 days earlier than the parent, which is a major advantage for harvesting before the onset of the rainy season in the respective areas. Even 30 years after their release these two varieties are still grown extensively in Thailand, covering 80% of the rice fields in north-eastern Thailand. According to the Bureau of Economic and Agricultural Statistics, during 1995-96, RD 6 was grown on 2,429,361 ha, covering 26.4% of the area under rice in Thailand, producing 4,343,549 tons paddy [5, 18], and in 2006 was still cultivated on an area of more than one million ha (S. Taprab, personal communication, July 2007). Thailand is the largest exporter of aromatic rice to the world market. Thus, the impact of the two rice mutant varieties is far beyond the farm gate with a major contribution to the export earnings. Between 1989 and 1998, the contribution of RD6 paddy was 4.76 billion US dollars, of milled rice 15.3 billion US dollars, and that of RD15 485.6 million US dollars for paddy, and 1.6 billion US dollars for milled rice. Hence, from 1989-98, the two varieties RD6 and RD15 yielded a total of 42.0 million tons paddy or 26.9 million tons milled rice worth 16.9 billion US dollars [5].

Other Asian countries

Induced mutations have also been widely used in many other Asian countries for breeding new varieties and in turn contributed to food security. Detailed information for Pakistan [19] and Vietnam [20] can be found elsewhere in this book.

In the Republic of Korea, sesame (*Sesamum indicum*) yield has been increased more than twice (from 283 kg/ha to 720 kg/ha) due to development and release of 15 improved determinate type, high oil content mutant varieties having phytophthora blight resistance and good

cooking quality. These mutants occupied 55% of the national acreage during the last two decades in Korea [21].

In Bangladesh, mutation breeding has resulted in the release of more than 40 mutant varieties belonging to more than 12 crop species. The Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, is the major center of mutation breeding, and has released 16 mutant varieties of pulses, 11 of oilseeds, seven of rice, and five of tomato. Rice mutant var. Binasail, Iratom-24, Binadhan-6, all planted in a cumulative area of 795,000 ha, and mungbean mutant variety Binamoog-5, cultivated in 15,000 ha as a summer crop, have contributed substantially towards food security in Bangladesh [Ali Azam, personal communication, April 2008].

In Myanmar, the rice mutant variety 'Shwewartun' was developed and released in 1975 after irradiation of 'IR5' seeds in 1970. The improvement in grain quality, seed yield, and early maturity of the mutant compared to its parent variety, led to its large-scale planting. Between 1989-1993, it covered annually more than 0.8 million ha - 17% of the 4.8 million ha area under rice in Myanmar [5].

During the past decade, Vietnam has become an icon for the success of mutation breeding. Though it used to import 2-3 million tons of food annually in the decade of 1970-1980, Vietnam exported 4.3 million tons of rice, becoming the world's second-largest exporter of rice. The wide use of high yielding crop varieties including dozens of mutant varieties contributed substantially to this transformation into food self-sufficiency. For example, the mutant rice variety VND-95-20, grown on more than 300,000 ha/year, has become the top variety in southern Vietnam, both as an export variety and in terms of growing area. More information about mutant varieties and their great importance for the food security in Vietnam today, can be found in another paper in this book [20].

EUROPE

Induced mutations have become an inherent component of many current plant varieties in Europe, particularly for barley and durum wheat. Mutation techniques are also widely used in the breeding of flowers and horticulture cultivars, although it is not the topic of this paper. A few examples are given here, while the situation in Sweden is available elsewhere in this book [22].

Czech Republic and Slovakia (former Czechoslovakia)

The Gamma-ray induced cultivar Diamant was officially released in Czechoslovakia in 1965. Diamant was 15cm shorter than the parent cultivar 'Valticky', and had an increased grain yield of around 12%. In 1972, 43% of 600,000 ha of spring barley in Czechoslovakia were planted under either Diamant or mutant cultivars derived from Diamant. Roughly estimated, the total increase in grain yield was about 1,486,000 tons. During the same year, the spring barley cultivars that had mutated Diamant's *denso* gene in their pedigree were grown all over Europe on an area of 2.86 million ha [23].

The high-yielding, short-height barley mutants Diamant and Golden Promise were a major impact on the brewing industry in Europe; they added billions of US dollars to the value of the brewing and malting industry. More than 150 cultivars of malting barley in Europe, North America, and Asia were derived from crosses involving Diamant [5].

Finland

Balder J, a high yielding barley mutant released in Finland, had higher yield, greater drought resistance, better sprouting resistance, and greater 1,000 kernel weight. Nearly 1 million kg of 'Balder J' seed were sold by Jokioinen Seed Center [24]. Oat stiff straw mutant cultivar Ryhti occupied up to 41% of the total area of oat in Finland during 1970-80. Another stiff straw oat mutant cultivar, Puhti, released in 1970, occupied 30% of the oat planting area in Finland. Many new varieties now grown are derived from crosses with these mutant varieties [25].

Germany

Trumpf, the best-known barley mutant cultivar obtained after crossing with cultivar Diamant occupied more than 70% of the barley planting area in Germany. The mutant had a yield increased by 15% and better disease resistance. Used extensively in crossbreeding, Trumpf became incorporated into many barley breeding programmes in a large number of countries [25].

Italy

Mutant cultivar Creso of durum wheat was grown in about one-third of the total area of durum wheat in Italy. During a period of 10 years, in Italy alone, an extra economic profit of 1.8 billion US dollars was obtained by growing this cultivar. Castelporziano and Castelfusano high-yielding durum wheat mutants had shorter culms and spike length, better resistance to lodging, but higher numbers of grain per spikelet. Planted in sizable areas, they contributed notably to the national economy of Italy. Both mutants were also used in extensive crossbreeding [5, 24].

NORTH AMERICA

In North America, the USA is one of the world pioneering countries in the exploitation of induced mutation for plant improvement and has had many extraordinary successes. Significant progress has also been reported from Canada, and more recently Mexico.

USA

Wheat: Stadler, a high-yielding wheat mutant released in Missouri, had early maturity, resistance to races of leaf rust and loose smut, as well as better lodging resistance. It was once grown on two million acres annually in the USA [24].

Barley: Luther, a barley mutant, had 20% increased yield, shorter straw, higher tillering, and better lodging resistance. About 120,000 acres were planted annually in three states of the USA - a gain of an estimated 1.1 million US dollars in one year. It was used extensively in cross-breeding and several mutants were released. Pennrad, a high yielding winter barley mutant was released in Pennsylvania, had winter hardiness, early ripening and better lodging resistance. It was grown on about 100,000 ha in the USA [24].

Beans: Sanilac, a high-yielding Navy pea bean mutant cultivar, developed after irradiation with X-rays and released in Michigan, was grown on more than 87,000 ha. Similarly, about 160,000 ha were planted with common bean cultivars Gratiot and Sea-way, developed likewise by cross-breeding with a Michelite mutant [5, 24].

Rice: The semi dwarf gene allele *sd1*, which was induced through Gamma-ray mutagenesis, has enabled the American version of the "Green Revolution" in rice. Details of the *sd1* allele and its contribution to the rice production in the USA (as well as in Egypt and Australia) are shown in this book [26].

Two grapefruit varieties, Star Ruby and Rio Red, both developed through thermal neutron mutagenesis [27], have become a widely grown variety during the past two decades. The fruits of both cultivars are sold under the trademark 'Rio Star'. 'Rio Star' grapefruit is currently grown on 75% of the grapefruit planting area in Texas. The development of the two radiation induced mutant cultivars is considered as the most significant breakthrough in grapefruit growing in Texas since the discovery of Ruby Red in 1929 [5].

Canada

Rapeseed-Canola (Double zero rapeseed): Canola is Canada's third most important grain export, after wheat and barley. Contribution of Canola cultivars to the Canadian economy has been outstanding. In 2000, Canada planted 5,564,000 ha under canola and earned 350.5 million US dollars. Mutant cultivars with low erucic acid and very low (more than 30µm/g) glucosinolates have been developed and released in Canada [5].

The strongest modification of oil composition with induced mutations has been the development and release of linseed cultivars of the 'linola' type in Australia and Canada. Zero is the low-linolenic acid genotype derived by EMS (ethyl methanesulphonate) mutagenesis of the Australian linseed cultivar Glenelg and recombination of two mutated genes [28].

Mexico

In Mexico, promotion of radio-induced mutation breeding started in 1974. Two new wheat varieties, 'Centaurus' and 'Bajío Plus,' were derived from 'Salamanca' seeds irradiated at 500Gy; they showed increased yield and tolerance to lodging. Two soybean varieties, 'Hector' and 'Esperanza,' were obtained by irradiation of seeds from variety 'Suaqui 86' at 150Gy. These new varieties exhibit an increased yield and reduction in dehiscence and lodging, being tolerant to white fly. 'SalCer' is another new soybean variety obtained through irradiation of seeds from line ISAEGBM₂ at 200Gy. Its improved traits are higher yields and increased height to first pod [29].

LATIN AMERICA

Argentina

Colorado Irradiado, a groundnut mutant with high yield and fat content, induced by X-rays, occupied more than 80% of the groundnut area (280,000 ha) in Argentina in the 1970s [Prina, A.R., Personal communication, August 2008]. Puita INTA-CL, a rice mutant with high yield and herbicide resistance, released in 2005, has occupied more than 18% of the rice growing area (32,400 ha) in Argentina since then [Prina, A.R., Personal communication, August 2008]. Also planted in Brazil, Costa Rica, Paraguay and Bolivia, this mutant variety has contributed significantly to these Latin American countries' economies and their food security.

Cuba

Rice: Attempts to obtain a rice mutant variety with good agronomical characteristics and salinity tolerance have been successful in Cuba. The first mutant released from *in vitro* mutagenesis using proton radiations in Cuba is 'GINES,' which shows the best performance under saline conditions, and has been successfully introduced in rural areas of Pinar del Río and Havana provinces [30].

Tomato: The very first tomato mutant released in Cuba, 'Maybel,' has shown very high performance under drought conditions and has been introduced in rural areas of different provinces of Cuba [31].

Peru

Mutation breeding has been very successfully used in breeding barley, the fourth most important food crop in terms of area in Peru. Centenario, a barley mutant with high yield (37% over the parent cultivar), earliness (18 days), higher protein (10.3%), better test weight and resistance to yellow rust, was released in 2006, is replacing the traditional cultivars of the central highlands of Peru, and contributes significantly to the food security of the country [32].

Kiwicha (*Amaranthus caudatus*) is a native and ancient crop of the Andean Region. Centenario (MSA-011), a mutant with high yield, earliness (45 days), tolerance to salinity, wide adaptability, better grain color and size, as well as higher market price, was released in 2006 and has covered 40% of the total Peruvian land dedicated to kiwicha crops [32].

AUSTRALIA

Rice: Nine rice varieties - 'Amaroo' (1987), 'Bogan' (1987), 'Echua' (1989), 'Harra' (1991), 'Illabong' (1993), 'Jarrah' (1993), 'Langi' (1994), 'Millin' (1995), and 'Namaga' (1997) - have been introduced in Australia. Rice mutant variety Amaroo has covered 60-70% of the rice cultivation area of Australia, and on average yielded 8.9 t/ha grain with a potential of 13.3 t/ha [33].

Lupine: Spontaneous mutation has been discovered and utilized in domestication of narrow-leafed lupine (*Lupinus angustifolius L.*). As the result of the domestication, lupine has become a dominant grain legume crop in Western Australia. Facing the new challenge of developing herbicide-tolerant cultivars, chemical mutagenesis has been used to create new tolerance to herbicide. The two lupine mutants (Tanjil-AZ-33 and Tanjil-AZ-55) are highly tolerant, six times more tolerant to metribuzin herbicide than the original parental cultivar Tanjil. This mutant Tanjil-AZ-33 is the most tolerant germplasm in narrow-leafed lupine. Both mutants also maintain the high yield and resistance to the disease anthracnose as cv Tanjil. These facts indicate that the mutation process has created tolerance to metribuzin in Tanjil, but has not altered Tanjil's yield capacity and anthracnose resistance. Induced mutation proves to be an effective tool in lupine improvement [34].

AFRICA

Egypt

As a result of the introduction of the two semi-dwarf mutant varieties, 'Giza 176' (1989) and 'Sakha 101' (1997) in Egypt, the average yield of rice in Egypt increased to 8.9 t/ha, compared with 3.8 t/ha in the rest of the world. Of these two, 'Giza 176' became the leading variety, with a potential yield of 10 t/ha [35].

Sudan

Mutation breeding in Sudan was effectively started about 20 years ago and covered crops like cotton, sugarcane, sesame, banana, tomato, groundnuts, and cereals. A banana mutant cultivar (Albeely) was released in the year 2003. Albeely excelled the yield of the existing cultivars by 40% and has better crop stand and fruit quality. Albeely is becoming popular and is widely preferred by farmers. A drought tolerant groundnut mutant (Barberton-B-30-3) and a number of promising mutants resistant to tomato yellow leaf curl virus (TYLCV) are being evaluated in multi-location trials, in preparation for their commercial release. Cotton germplasm has been enriched with a number of useful mutants carrying resistance for bacterial blight and fusarium wilt disease, in addition to mutants for weak fiber attachments, high ginning out turn, and lint percentage. These mutants are being used in the breeding programme, and promising lines are under field evaluation for release [36].

Ghana

Over two decades of application of induced mutation techniques toward crop improvement in Ghana have led to the production of improved mutant varieties in two crops. In cassava (*Manihot esculenta* Crantz), irradiation of stem cuttings using gamma irradiation resulted in the production of "Tek bankye," a mutant variety with high dry matter content (40%) and good poundability from the parental line, which was a segregant of a hybrid between the Nigerian landrace Isunikaniyan (ISU) and the breeder's line TMS4(2)1425, both from IITA, Nigeria. Similarly, irradiation of vegetative buds of 'Amelonado' (P30), 'Trinitario' (K.5), and 'Upper Amazon' (T85/799) cocoa varieties resulted in the production of a mutant variety resistant to the Cocoa Swollen Shoot Virus (CSSV). Multi-location on-farm trials of the mutant line indicate significant increases in yield for farmers, without symptoms of the disease [37].

Perspectives

World food security deteriorated very sharply in the 1960's when developing countries like India, Pakistan, and Indonesia were desperately short of food grains. Fortunately, agricultural scientists responded with a new production technology, which has popularly been described as "Green Revolution Technology." This helped to avoid large-scale starvation for around 40 years. However, the food security problem has again seen a major deterioration in the last few years; food prices are rising sharply and once again the poor people of the world are threatened

with serious malnutrition. The underlining causes that drove to food security deterioration, i.e. rising fuel and fertilizer prices, climate change related erratic rain falls, sudden and severe drought conditions, excessive floods, divert of food grains into bio-fuel production, will remain for the years to come. Food security will even get worse since population is still growing while no significant expansion of arable lands is foreseen. FAO estimates that world food production should increase by more than 75% in the next 30 years to feed about eight billion people by 2025 [38]. Therefore, a new "Green Revolution" is desperately needed to solve the food security issue in the years to come.

The massive advent of plant molecular biology is anticipated to provide a sound solution to further increase food production by both increasing yield potential and stability. In this regard, induced mutagenesis is gaining importance in plant molecular biology as a tool to identify and isolate genes, and to study their structure and function. Several papers in this book report the progress being made in this area. Recently mutation techniques have also been integrated with other molecular technologies, such as molecular marker techniques or high throughput mutation screening techniques; mutation techniques are becoming more powerful and effective in breeding crop varieties. Mutation breeding is entering into a new era: molecular mutation breeding. Therefore, induced mutations will continue to play a significant role for improving world food security in the coming years and decades.

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Eighty Years of Scandinavian Barley Mutation Genetics and Breeding

U Lundqvist

Abstract

In 1928, the Swedish geneticists H. Nilsson-Ehle and Å. Gustafsson started on their suggestion experiments with induced mutations using a diploid barley species. The experiments started with X-rays and UV-irradiations, soon the first chlorophyll mutations were obtained followed by the first 'vital' mutations 'Erectoides.' Several other valuable mutants were considered: high-yielding, early maturity, lodging resistance and with changed ecological adaptation. Soon the X-ray experiments expanded with different pre- and after-treatments, also using other types of irradiation, such as neutrons, positrons etc., and finally with chemical mutagens, starting with mustard gas and concluding with the inorganic sodium azide. The research brought a wealth of observations of general biological importance, high increased mutation frequencies, differences in the mutation spectrum and to direct mutagenesis for specific genes. This Scandinavian mutation research was non-commercial even if some mutants have become of some agronomic value. The peak of its activities was during the 1950s, 1960s and 1970s. Barley has been the main experimental material, but also other species were included in the programme. Over the years a rather large collection of morphological and physiological mutations (10,000 different mutant genes) with a broad variation were collected and several characters have been analyzed in more detail genetically and with regard to mutagen specificity. Most effort has been made on the *Early maturity* mutants, the *Six-row (hexastichon)* and *Intermedium* mutant group, the *Surface wax coating: Eceriferum (Waxless)* mutants, *Dense spike* mutants and others.

The first mutation experiments

Swedish research on induced mutations started in a small scale at Svalöf 80 years ago, initiated by the eminent Swedish geneticists H. Nilsson-Ehle and Å. Gustafsson. Already in 1927, the North American geneticist and later Nobel laureate, H.J. Muller, using successful experiments could show that ionizing radiation could increase the mutation frequency in the fruit fly *Drosophila* [1, 2]. He drew the conclusion that induced mutations were similar to spontaneous mutations forming the basis for natural selection and evolution. Soon, in 1930, the equally famous American geneticist and plant breeder, L.J. Stadler [3], published data on induced mutations in several species of cultivated plants which he interpreted with much pessimism. In his opinion, no practical progress could be expected from artificial gene changes. But Nilsson-Ehle and Gustafsson did not share this pessimism and on Gustafsson's suggestion experiments were initiated with induced mutations in plant material.

The first treatments with X-rays and ultraviolet irradiation were commenced in barley, using the Svalöf cultivar 'Gull.' Instead of measuring exact dosages of radiation, different durations of radiations were applied. Also different types of pre-treatments were tested since it was known from Stadler's investigations that the mutation frequency increases if the seeds are soaked in water before irradiation. The first chromosome

aberrations were observed, mainly chromosome fragmentations, fusions and translocations [4]. The first genotypical changes in the seedlings, chlorophyll mutations, most of them sublethal, occurred. Three distinct main categories were established: *albina* seedlings, *viridis* seedlings, a very heterogeneous group, and rare mutations (*xantha*, two-coloured, striped and zoned). These chlorophyll mutations were a useful material for laboratory studies and were the first indications of how successful the treatment was. The mutation frequency was calculated according to the "spike progeny method" introduced by Gustafsson, and served as the standard method for measuring the induced mutagenic effects [5, 6].

Very soon, in the mid-1930s, the first viable mutations appeared and already at that time it was possible to distinguish two sub-groups: *Morphological* and *Physiological* mutations. The most common group of viable mutations at that time consisted of the so-called *erectoides* mutants that are characterized by typical compact or dense spikes in contrast to the *nutans* spike of most barley cultivars. In the following years many of the mutants produced were considered extremely valuable for future theoretical genetic studies and for breeding. Several of them are worth mentioning: high-yielding, early maturity, tillering capacity, straw-stiffness, seed-size, seed-color, changed spike formation and others [7, 8].

The Swedish group for theoretical and applied mutation research

The results from these early experiments looked so promising, even for plant breeding, that in 1940, the Swedish Seed Association at Svalöf started to support this research with funding from the Swedish milling industry. This rendered it possible to extend the experiments considerably. In addition, other species such as wheat, oats, flax, peas, faba beans and oil crops were included in the programme, and it became possible to integrate theoretical and practical results. In 1948, the Wallenberg Foundation incorporated mutation activities into its research programme, and a group of specialists were gathered to carry on the research work on a wider front. Finally, in 1953, at the instigation of the Swedish Government the 'Group for Theoretical and Applied Mutation Research' was established, with the aim of studying basic research problems in order to influence and improve the methods for breeding of cultivated plants. The Agricultural Research Council had provided funding for most of the Mutation Group's scientific activities approved by the Swedish Parliament. Its peaks of activities were during the 1950s, 1960s and 1970s. Barley was used as main model crop since it is a diploid self-fertilizing species, easy to handle, gives a sufficiently large progeny from a single plant and outcrosses only rarely [9, 10].

Applying different mutagenic treatments

X-irradiation on dry seeds was used as a standard method for studying the mutation process, but soon other types of irradiation such as γ -rays (acute and chronic), neutrons (fast and thermal), electrons, protons, α -rays from radon, β -rays from Phosphorus 32 and Sulphur 35 were included in the experiments. The application of pre-treatments with different soaking times of the seeds, both before and after irradiation

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was studied. Not only the water content of the seeds was an important trait in relation to radiation sensitivity, but also different environmental conditions [11, 12]. The two irradiation types, sparsely versus densely ionizing radiation, were compared in the following properties: (1) the number of chromosome disturbances in the germinating seeds, (2) field germination, (3) number of mature harvested plants, (4) the mutation rate determined from the number of various chlorophyll-deficient mutants, and (5) different types of vital mutants determined on field material in the second generation. When comparing the two irradiation types it can be summarized as follows: The injurious action of neutrons differs from that of X-rays in several respects. The seeds are 20-30 times more sensitive in neutrons than to X-rays and germinating seeds are two to three times more sensitive to neutrons than dormant seeds. Neutrons are approximately 10 times as effective as X-rays in producing chromosome disturbances and about 50-100 times more effective in increasing the mutation rate in the second generation. Neutrons produce relatively more chlorophyll mutations than X-rays. Observations also showed that while X-irradiated seeds die at a very early stage of development, the neutron-treated seeds which received a lethal dose often start germination, not dying until cell divisions become of critical importance for their further growth [13, 14].

Already in the mid-1940s chemical mutagenesis started to be included in experiments together with irradiation. The idea was to influence not only the mutation rate but also the types of mutations. The real work on chemical mutagenesis in crop plants began with the effects of mustard gas followed by many different compounds such as various alkylating and oxidizing agents, epoxides and epimines, purines, organic sulphates and sulphonates, nitroso compounds, purine and acridine derivatives and many others [15-23]. Finally, in the mid-1970s, the first experiments were started with the inorganic chemical mutagen 'Sodium azide' that in Swedish experiments was mostly used for isolation of viable mutants for practical agronomical purposes [10, 24, 25]. For chemical mutagens the mutation frequency increased rapidly up to 80%; they were 20 times more effective than irradiation. Significant differences between the actions of ionizing radiation and chemical mutagens were demonstrated. In this respect, neutrons and sodium azide form two extremes: neutrons induce a relatively large number of chromosome and chromatid changes, whereas sodium azide primarily causes gene mutations at the nucleotide level. Differences in the mutation spectrum were noticed especially with regard to chlorophyll mutants as they were studied most intensively. Neutrons induce a higher rate of albina seedlings than X-rays and chemical mutagens. The chemical mutagens are superior in inducing the large heterogeneous group of viridis seedlings. Also in some morphological mutation groups and mutants useful for plant breeding it was possible to observe differences in the mutation spectrum. The aim was to control the direction of mutagenesis [10, 26].

The Swedish mutation research was non-commercial, despite that some mutants have been used in practice – directly or after recombination breeding [27]. The mutation programme brought a wealth of observations of general biological importance: chronology of chromosome reproduction, sensitivity of different mitotic stages, the importance of heterochromatin, mutations in polyploids, the variation of irradiation sensitivity in different species and competition between various elements in plant tissues.

The Swedish collection of barley mutants

Genetic diversity is an important feature in plant breeding and the breeder can use the artificially induced mutants for further improvement of his cultivars. A methodical work will sooner or later lead to positive results.

Over the years a large collection of morphological and physiological mutations (10,000 different mutant alleles) with a broad variation range have been brought together and have been genetically and agronomi-

cally studied. They consist of 10 main categories with 116 different subtypes (Table 1). So far, about half of these mutants have been analyzed genetically in more or less detail, but they form only a minor part of the range of mutant types. This collection forms an outstanding material for investigations within radiobiology, genecology, gene physiology, ultrastructural research and plant biochemistry, and physiology. It is a major source for future gene mapping and is valuable for molecular genetical analyses of cloned mutant genes. This Swedish collection is unique since all the alleles of the investigated genes are conserved at the Nordic Genetic Resource Center (former Nordic Gene Bank) and available for research and breeding. The mutant groups shown in the table below were studied in detail genetically and with regard to mutagen specificity. These studies have increased our knowledge of the mutation process and the genetic architecture of the different characters. In this presentation a few of these groups are presented in more detail (Table 2) [10, 28-31].

Table 1. Survey of the main mutant categories

1.	Changes in spike and spikelets
2.	Changes in culm length and culm composition
3.	Changes in growth type
4.	Changed kernel development and formation
5.	Physiological mutants
6.	Awn changes
7.	Changes in leaf blades
8.	Changed pigmentation
9.	Different chlorophyll development
10.	Resistance to powdery mildew

Table 2. Survey of the genetically investigated Scandinavian mutant groups

Mutant group	Number of alleles	Number of loci
Praematurum (Early maturity)	195	9
Erectoides	205	26
Breviaristatum (Short awns)	184	19
Eceriferum (Waxless, Glossy)	1580	79
Hexastichon (Six rowed spike)	65	1
Intermedium	80	10
Lemmlike glumes (Macrolepis)	40	1
Third outer glume (Bracteatum)	10	3
Calcaroides	21	5
Anthocyanin mutants	766	31
Liguleless (Auricleless and Exligulum)	24	2
Albino lemma (Eburatum)	5	1
Orange lemma (Robiginosum)	7	1
Mildew resistance	77	several
Chlorophyll synthesis and chloroplast development	357	105

Praematurum (early maturity) mutants

The demand for early cultivars has increased why earliness has become an important goal for Swedish plant breeding. Already in the 1940s, it was found that maturity in barley could easily be changed by X-rays, in either direction of both increased earliness and increased lateness. The time of heading was chosen as a safe character for the selection of induced early mutants, but early heading and early ripening are characters where environmental influences, especially climatic conditions may hamper a safe classification [32].

Over the years, about 1,250 different early maturity mutants have been isolated and studied after various mutagenic treatments. The mutants can be grouped into three categories according to their heading and maturity time with a variation between one and 10 days. Long term studies made it possible to establish nine *mat* loci among 195 localized mutants (Table 3) [10].

Table 3. Distribution of the early maturity mutants to the 9 *mat* loci

Locus	<i>mat-a</i>	<i>mat-b</i>	<i>mat-c</i>	<i>mat-d</i>	<i>mat-e</i>	<i>mat-f</i>	<i>mat-g</i>	<i>mat-h</i>	<i>mat-i</i>	Total
Mutants	85	49	31	2	9	7	4	2	6	195

The different loci in general show quite distinct phenotypic characters. The mutations selected for earliness also change other properties of agricultural value. Significantly shorter straw with lower internode number is found in the extreme early mutant loci, *mat-a*, *mat-b* and *mat-c*. Mutants of locus *mat-a* are generally more resistant to lodging than mutants in locus *mat-b*. Among these loci, *Praematurum* (*mat-a.8*) mutant, a drastic early mutant, heads 8 to 10 days earlier than its mother cultivar 'Bonus.' It was approved and released as a commercial Swedish cultivar under the name of 'Mari' in 1960, and was intended to replace early Swedish six-row cultivars. It was widely grown, as far north as Iceland and it was included in the breeding programme at Cymmit, Mexico [33]. Not until the mid-1960's it was found that *mat-a.8* had a special property that distinguished it from the 'Bonus' parent, namely, a profound change in the photo- and thermoperiod reaction, making it heading and seed fertile also at eight hours of daylight (short-day tolerant). During the 1960's, large phytotron experiments were carried out in Stockholm under different photoperiod conditions to compare different mutants and cultivars [34-36]. Later, when labour costs got too expensive, a darkening arrangement, using a special plastic tissue, was used in ordinary glasshouses with natural light lasting for eight hours. This type of arrangement was used for many years for identifying short day neutral mutants. It was possible to distinguish three genotype categories under the extreme short day conditions of eight hours of light: (1) genotypes with complete and early heading and good seed set; (2) genotypes with incomplete and late heading and seed set; and (3) genotypes that never headed remaining in a purely vegetative often luxurious stage. The mutants in *mat-c* and *mat-e* loci, are characterized by delayed heading and thus, a less pronounced short-day neutrality. The mutants in all other *mat* loci are long-day adapted like the parent cultivars [37]. Concerning the mutagenic treatments, there is a concentration of short-day adapted mutants under sulfonate treatments whereas the long-day adapted cases seem to accumulate when ethylene imine is applied. Other observations indicate that sodium azide is less efficient in producing day-length neutral mutants.

Six-row (*hexastichon*) and *intermedium* mutants

Genetic variability in barley has been of great importance and has long been studied in great detail. The Russian geneticist N.I. Vavilov felt it necessary to explore the total genetic diversity of crop plants throughout the world as well as diversity of related wild species. Barley is one of the oldest cultivated crops [38]. The number of rows of the spikelets is a key character in inferring the origin of barley. For at least 100 years, it has been discussed whether the progenitor of cultivated barley was six-rowed, two-rowed or both. Recently, the two-rowed progenitor hypothesis was supported by archeological specimens showing the existence of domesticated two-rowed remains that were older than six-rowed. The six-row (*hexastichon*) and *intermedium* mutants affect the development of the lateral spikelets with genetic interaction leading to synergistic enhancements. This research has given an insight into rather complex genetics of kernel rows in barley. Normal two-row barley carries, on opposite sides of the spike, central spikelets, with two reduced, sterile

lateral spikelets. The two-row barley is able to produce six-row barley in a single mutational step. These mutants have well developed lateral spikelets, fully fertile and with long awns. All the 45 isolated cases have been localized to only one locus, the *hex-v* (*vrs1*), located in the long arm of chromosome 2H [39]. Recently this six-rowed spike 1 (*vrs1*) gene was cloned by the Japanese research group (Komatsuda, *et al.*, 2007). It is indicated that it is a homeodomain-leucine zipper I-class homeobox gene. Expression of the *Vrs1* was strictly localized in the lateral spikelet primordial of immature spikes and suggests that the VRS1 protein suppresses development of lateral spikelets [40].

But two-row barley may also produce mutants with spike development intermediate between the two-row and the six-row states. These mutants have enlarged lateral spikelets with characteristic awn and kernel development, not only among mutants, but also depending on environmental conditions. A total of 126 such *intermedium* mutants have been isolated and 103 of them have been localized to 11 different *int*-gene loci and studied in more detail (Table 4) [41].

Table 4. Distribution of the *intermedium* mutants to the 11 *int* loci

Locus	-a	-b	-c	-d	-e	-f	-h	-i	-k	-l	-m
Number	33	3	23	21	14	1	4	1	1	1	1

The *hexastichon* (*hex-v*) mutants have six-rowed spikes with fully fertile, well developed, and long-awned lateral spikelets and thus resemble normal six-rowed barley. The *intermedium spike-d* (*Int-d*) mutants produce sterile or partially fertile lateral spikelets with variable awn length which appears intermediate to those of two-rowed barley. Allelism of these mutants showed that these two loci are closely linked to one another on chromosome 2HL and are more or less dominant. Komatsuda, *et al.* (2007) showed that the *Int-d* mutants are the same gene and morphological changes in the mutants can be attributed to changes in the *Vrs1* gene [40]. All other 10 *int* loci are recessive and showed independent inheritance. Both radiation types and most of the used chemical mutagens have been involved but no gene preference to the type of mutagen applied has been found [10].

Surface wax mutants: *Eceriferum* (*waxless*)

Presence of wax coating reduces evaporation of water from the plant and helps protect it against pathogens. Most surface wax mutants, the *eceriferum* and *glossy* loci affect the presence and type of epicuticular waxes on the leaf blades and sheaths, culms and spikes. When the wax coating is completely absent, various organs appear as a bright, glossy green color. Cooperation between Swedish and Danish researchers has made this mutant type probably the best known character complex of any cultivated plant. The mutants have been gene localized, their influence on yield studied, electron microscopy and biochemical analyses done, different loci mapped in chromosomes, and their reactions to various climates studied in the phytotron. Phenotypically three different organs of the barley plant were studied in regard to wax coating and composition and led to five phenotypic categories: spike and leaf sheath, spike and leaf sheath partially, spike, leaf blade, and spike, leaf sheath and leaf blade [42, 43]. A total of 1,580 such *eceriferum* mutants have been localized to 79 loci, 78 of them are recessive and one is dominant. Seven types of mutagenic treatments have been applied. It is obvious that different loci show markedly differing mutagen specific reactions. (1) There are particularly large mutagenic differences between chemicals and ionizing radiation, especially neutrons; (2) No significant differences among various kinds of organic chemicals can be established; (3) There are significant differences between organic chemicals and sodium azide; (4) The combined treatment (sulfonate + X- or γ -rays) does not differ from treatment with sulfonates alone, but differs from the treatment with exclusively X-rays. No difference to sodium azide can be demon-

strated; (5) Sodium azide differs strongly from X-rays and still more from neutrons; (6) There are clear differences between the two kinds of treatments with ionizing radiation; sparsely ionizing X-rays and densely ionizing neutrons having different effects on the target DNA molecule. In summary, the wealth of alleles distributed on a large number of *cer* loci has provided important insights into the mutation process. Obviously, different gene loci have different mutabilities. It is equally obvious that different loci show markedly differing mutagen specific reactions. These insights into the mutation process combined with knowledge of the localization of the different genes in the genome will add to our understanding of the mechanisms of mutagenesis and the organization of the eukaryotic genome [10, 44].

Dense spike mutants

The dense spike (*erectoides*) mutants were the first of the viable mutants induced by irradiation and the most commonly induced morphological changes. They are characterized by compact, dense spikes, implying that the spike rachis internodes are shorter than in the mother strain. They generally possess a very stiff and often short straw. The first uppermost internode of the culm is generally longer than in the mother cultivar and the basal ones being shorter. In all, about 1,270 such mutants have been isolated and studied intensively. Twenty-six gene loci could be established among 205 investigated mutants. Most of the loci have distinct phenotypic characteristics, one of them is dominant and all the others recessive. Several loci have been mapped spread over the seven barley chromosomes. Differences in the mutation spectrum could be noticed. Three of these loci could be identified as mutagen specific, where more than 80, 70% and 50%, respectively, of the alleles were induced by irradiation [45-48]. Most of these *erectoides* mutants are fully viable and promising from a practical point of view. The most outstanding of these mutants is *erectoides* 32 in locus *ert-k* that became released as a new cultivar 'Pallas' in 1958 [49].

Breeding aspects

Since work with artificial induction of mutations began, it was evident that mutation programmes should be regularly included in breeding programmes of crop plants. The application of mutation research in plant breeding was the most important stimulus. It was shown already in the 1950s and 1960s that the work at Svalöf can be used as an example how mutation breeding can be employed in a crop improvement programme. The main interest was focused on macromutations [50]. Both simple and rather complex characters such as straw-stiffness, earliness, higher yields, protein content and disease resistance are of interest. Not only new direct mutants, but also the indirect use of induced mutations was applied. In the latter case breeding work tried to change modifying systems by crossing mutants with various established cultivars and selecting the best recombinants homozygous for the mutations. In the Swedish programme, this use of macromutations in conventional cross-breeding programmes has proved to be more successful than recurrent mutagenic treatments [51].

Through the joint work with barley breeders and other scientists at Svalöf, a rather large number of mutant cultivars of two-row barley were registered as originals and commercially released. Two of these cultivars 'Pallas', a straw-stiff, lodging resistant and high-yielding *erectoides* mutant, and 'Mari', an extremely early, photo- and thermo-insensitive mutant, were produced directly by X-irradiation. All other cultivars derive from crosses and backcrosses, where the original breeding material was based on three primary high-yielding Swedish X-ray mutant cultivars: 'Pallas', 'Sv 44/3', both extreme lodging resistant, and 'Mari', extremely early. A series of cultivars obtained after crossing, (Table 5) were tested and found to be agriculturally suitable to various parts of Scandinavia and other parts of the world. The aim of this work was to demonstrate that original mutant materials can be used successfully in

recombined breeding programme in the hands of skilful breeders. It has set a positive trend, in fact, as positive and progressive as any other method of plant breeding [52]. Today, with modern technology, different methods ought to be used together, adding to the results of ordinary crossing and selection.

Useful mutations in barley include a wide range of economically important characters that influence morphological as well as the physiological and biochemical properties. The use of the mutation method is of importance in providing a detailed understanding of the genetic composition of the barley genome, especially if combined with detailed chromosomal and genetic analyses of linkage and biochemical studies of the DNA constitution and the amino acid composition.

Table 5. Survey of induced barley mutants and their derivatives, approved and released at Svalöf (after Gustafsson, 1986)

Parent strains	Derivates
	(1) Primary mutant varieties
'Gull'	44/3: extremely lodging resistant
'Bonus'	'Pallas' (ert-k.32) approved 1958
'Bonus'	'Mari' (mat-a.8) approved 1960
	(2) Varieties approved
Mutant crosses	
Herta x Pallas	'Hellas', approved 1967
Domen x Mari	'Kristina', approved 1969
Mari x Monte Cristo	'Mona', approved 1970
Birgitta x Mari	'Eva' and 'Salve', approved 1973 and 1974 respectively
44/3 x Birgitta	'Gunilla', approved 1970
(Birgitta x Mari) x Gunilla	'Pernilla', approved 1979
	(3) Varieties approved
Complex mutant crosses	
(Pallas x Triple awn lemma) x Pallas ^{bc}	'Visir', approved 1970
(Triple awn lemma x Pallas ^a) x Hellas	'Senat', approved 1974
Å 61657 x (Mari ^c x Triple awn lemma)	'Troja', but withdrawn 1981
Kristina x (Hellas ^a x (Pallas ^b x Rupee))	'Jenny', approved 1980
Lofa x [Å 6564 x (Mari ^{bc} x Multan)]	'Lina', approved 1982

In conclusion, Å. Gustafsson's words from his last paper in 1986 [53] are summarized as follows: "Induced mutations in the hands of skilful breeders will be an important future tool in progressive plant breeding. This will be even more so when the chemistry of the gene has been studied more thoroughly. Genetic instruments of artificial selection will increase the power and capacity of the plant breeder. It seems rather strange that also today there is a certain negative attitude towards the use of mutations in plant breeding or in most experiments concerning the general evolutionary theory. Such negative ideas are often associated with the view that mutationists ignore the natural sources of genetic variability and oppose the breeding value of primitive biotype collections."

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The Induced *sd1* Mutant and Other Useful Mutant Genes in Modern Rice Varieties

J N Rutger

Abstract

Induced mutation was accelerated in the USA with the release in California in 1976 of Calrose 76, the nation's first semidwarf table rice variety. Success was due not only to induction of mutants but also to their evaluation and integration into cross-breeding programmes. Thus the evaluation of Calrose 76 showed that its *sd1* gene was allelic to *sd1* in the indica Green Revolution varieties DGWG, TN(1) and IR8, and that semidwarfism conferred a yield advantage of 14% over the 6mt/ha yield level of the tall japonicas. Immediate integration of the Calrose 76 source of semidwarfism into cross-breeding has resulted in 25 semidwarf varieties that trace their ancestral source of semidwarfism to Calrose 76: 13 in California, 10 in Australia, and 2 in Egypt. Calrose 76 ancestry also appears in the pedigrees of numerous additional California cultivars derived from crossing the Calrose 76 source with the IR8 source of semidwarfism. In the late 1990s 12 semidwarf mutants were induced in tall tropical japonica varieties at the Dale Bumpers National Rice Research Center in Arkansas. The semidwarfing gene in each of these 12 germplasms was found to be nonallelic to *sd1*. Although selected for productivity, none of the 12 consistently showed yield increases typical of *sd1* sources. The *sd1* source, whether from induced mutation or from the indica source, is truly associated with enhanced productivity. Other induced mutants were found for early flowering, low phytic acid, giant embryo, and marker genes such as gold leaf and extreme dwarfism. The early flowering mutants were recovered in temperate japonicas, in tropical japonicas, and most recently in indicas. The early flowering indica mutants are quite interesting since they provide high-yielding or blast disease-resistant indica germplasm which will mature in the USA.

Introduction

The author's experiences with induced mutation for rice improvement have had two parts: First, from 1970-1988 as a Research Geneticist with the U. S. Department of Agriculture, Agricultural Research Service (USDA-ARS) at Davis, California, working on temperate japonica rice; and second, from 1993-2005 as Director of the USDA-ARS Dale Bumpers National Rice Research Center (DB NRRC) at Stuttgart, Arkansas, working on tropical japonica and on indica rice. Following a final period as Chief Scientist at the DB NRRC the author retired in January 2007 and moved back to California. Throughout his career the author has concentrated on selecting agronomically useful mutants such as semidwarfism and early flowering, with occasional detours into male steriles and marker genes as genetic tools. Keys to the success of the work have been, first the induction of mutants in very good varietal backgrounds, second their evaluation both agronomically and genetically, and third, immediate integration into conventional cross-breeding programmes by rice breeding colleagues.

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Figure 1 The induced mutant Calrose 76, released in 1976, was the first semidwarf table rice variety in the USA. It was about 25% shorter than its parent and the closely related tall check variety CS-M3 [7]

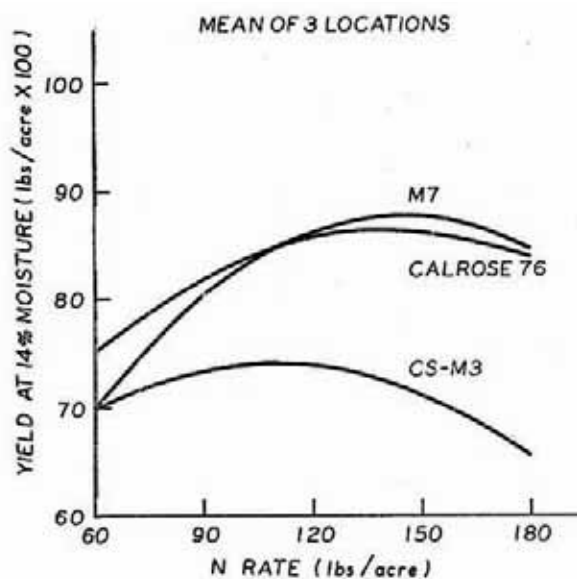


Figure 2 Averaged over nitrogen fertilizer rates from 60 to 180 lb/acre (67 to 202 kg/ha), the two semidwarf varieties Calrose 76 and M7 yielded 14% more grain and 13% less straw than the tall cultivar CS-M3 [2]

Temperate japonicas

Induced mutation work in the early 1970s resulted in the induction and direct release of Calrose 76, the first semidwarf table rice variety in the United States [1]. The work was a cooperative effort involving an IAEA-sponsored visiting scientist from Taiwan, Dr. Chao-hwa Hu, several

University of California Scientists, and the author. Following the induction of the semidwarf mutant, it was evaluated in yield trials where it was shown to be about 25% shorter than its parent and the closely related tall check variety CS-M3 (Fig. 1), and to produce 14% more grain and 13% less straw than the tall check (Fig. 2) [2]. Genetic evaluation showed that the semidwarfing gene in Calrose 76 was allelic to *sd1* from DGWG [3]. However, the greatest application of Calrose 76 was its integration into cross-breeding efforts. For example, the semidwarf, early maturing variety M-101 [4] was developed from the pedigree CS-M3/Calrose76//D31, where D31, another mutant from Calrose [5], contributed early maturity. The most popular Calrose 76 derivative semidwarf was M7, from the cross Calrose 76/CS-M3 [6]. Altogether, Calrose 76, usually through the derivative glabrous leaf variety M7, has served as the ancestral source of semidwarfism for numerous varieties: 13 in California [7] (Table 1), 2 in Egypt [18], and 10 in Australia (R. Reinke, Rice Breeder, Yanco Agricultural Institute, personal communication, December 21, 2005).

Table 1. California varieties for which Calrose 76 served as the ancestral source of semidwarfism

Variety	Year	Pedigree	Reference
Calrose 76	1976	Induced mutant of Calrose	[1]
M7	1978	Calrose 76/CS-M3	[6]
M-101	1979	CS-M3/Calrose 76//D31	[4]
M-301	1980	Calrose 76/CS-M3//M5	[8]
S-201	1980	Calrose 76/CS-M3//S6	[9]
M-302	1981	Calrose 76/CS-M3//M5	[10]
Calmochi-101	1985	Tatsumimochi//M7/S6	[11]
S-101	1988	70-6526//R26/Toyohikari/3/M7/74-Y-89/SD7/73-221	[12]
M-103	1989	78-D-18347/M-302	[13]
S-301	1990	SD7/730221/M7P-1/3/M7P-5	[14]
S-102	1996	Calpearl/Calmochi-101//Calpearl	[15]
Calhikari-201	1999	Koshihikari/(Koshihikari/S-101)*2	[16]
Calamylow-201	2006	Induced low amylose mutant of Calhikari-201	[17]

Calrose 76 ancestry also appears in the pedigrees of many additional California varieties resulting from crosses between the Calrose 76 source and the indica sources IR8 or DGWG (K.S. McKenzie, Director of California Cooperative Rice Research Foundation (CCRRF), (personal communication, August 22, 2005). Molecular technology now makes it possible to determine exactly which parent contributed the semidwarf allele gene in such semidwarf x semidwarf crosses. For example, the most successful variety in California for the last two decades, M-202 [19], derived from crossing the Calrose 76 source with the IR8 source, was recently shown to carry *sd1* from IR8, while S-101, another variety resulting from crossing the two sources, carries *sd1* from Calrose 76 (T. H. Tai, Rice Geneticist, Davis, California, personal communication, October 17, 2006). Another recent report confirms that M-202 carries *sd1* from IR8 rather than from Calrose 76 [20].

A measure of how extensively the CCRRF rice breeders have used mutants in their programme can be gleaned from perusal of their 26 mutant or mutant-derived varieties in the mvgs.iaea.org database [21]. As well as using the ancestral Calrose 76 *sd1* source of semidwarfism in cross-breeding the CCRRF has made direct releases of four independent mutant varieties:

- Calmochi-201, a waxy endosperm mutant of the variety S6 [22].
- M-401, a semidwarf mutant of the premium grain variety Terso [23].
- M-203, an early maturity mutant of M-401 [24].
- Calamylow-201, a speciality low amylose (about 6%) mutant of Calhikari-201 [17]. Calamylow-201 thus has two mutant genes,

sd1 from its Calhikari-201 parent, and the newly induced gene for low amylose.

The CCRRF also has developed aromatic varieties through use of basmati semidwarf mutants [25]:

- A-201, includes a basmati semidwarf, PI457920, in its parentage [26]
- Calmati-201, includes another basmati semidwarf, PI457918, in its parentage [27]
- Calmati-202, another basmati semidwarf, with A-201 as a parent (K.S. McKenzie, Director of California Cooperative Rice Research Foundation, personal communication, June 1, 2008).

Tropical japonicas

Upon becoming Director of the DB NRRC in Arkansas in 1993, the author launched a programme to induce semidwarf mutants in Arkansas tall, tropical japonica, varieties, since at that time no semidwarf varieties had been released in Arkansas. Inheritance studies showed that each of the 12 mutants induced had a recessive gene for semidwarfism [28, 29, 30]. When crossed with a known source of the Calrose 76 semidwarfing gene, each of the new semidwarfs was found to be nonallelic to *sd1*. Intercrosses among the 12 sources were not done, so it not known how many separate genes were involved. Each semidwarf was selected to be competitive with its tall parent, but none consistently exceeded the yield of its parent, as had been the case with *sd1* in California. Although not useful for direct release as mutant varieties, the 12 new semidwarfs were released as improved germplasm to interested breeders and other scientists.

Other tropical japonica mutants induced during the Arkansas period included: the KBNT lpa 1-1 low phytic acid mutant, with a 45% reduction in phytic acid [31]; the LGRU *ef* early flowering mutant [32]; and two dominant and one recessive genetic male sterile mutants [33]. The low phytic acid mutant also was crossed to a related variety carrying the goldhull (*gh*) gene to produce goldhull low phytic acid germplasm (GLPA) that could be identified by its goldhull marker gene in the field, in the farm truck, and in the grain elevator [34].

Indicas

By the mid-1990s, it was well established that indica germplasm, if sufficiently early in maturity, significantly outyielded the tropical japonicas in the southern USA [35]. Therefore the author began crossing a very early, bold grain, variety from China, Zhe 733, with six improved indica germplasms from IRRI, resulting in the release of nine indica germplasms, indica-1 to indica-9, with yield and maturity similar to southern varieties and grain quality approaching USA long grain standards [36]. Grain quality of the IRRI parents was almost identical to USA long grains, but the IRRI parents were about a month too late in maturity for USA environments. Therefore a programme was initiated to induce early maturity in the IRRI indicas, resulting in the release of six early flowering germplasms, indica-10 to indica-15 [37, 38]. Typical of these was indica-12, which was 28 days earlier than its indica parent, making it almost as early as local tropical japonica varieties (Fig. 3). Another indica of interest was the famous blast resistant variety from Colombia, Oryzica llanos 5, which also was a month too late for Arkansas. Therefore, early maturity mutants were induced in this variety, resulting in the release of three improved germplasms, indica-16 to indica-18, which were 24 to 36 days earlier than the parent and retained its blast resistance [39].

Other indica mutants induced during the Arkansas years included: the Guichao 2 *eui* mutant [40], which is allelic to the temperate japonica *eui* mutant found in California some 24 years earlier [41]; four indica genetic stocks, for apoptosis, narrow leaf, extreme dwarf, and gold leaf [42]; and four more genetic stocks, including: the long grain giant embryo mutant GSOR 25; a population segregating for albinos, GSOR 26, for elementary school teaching demonstrations; and two double-dwarf mutants, GSOR 27 and 28, which are 15 to 20cm shorter than their respective single-dwarf parents [43].



Figure 3 The induced early flowering mutant *indica-12* (right) was 28 days earlier than its IRR1 parent (left), making it useful for the USA since the IRR1 parent is about a month too late when grown in the USA [7].

A japonica/indica mapping population of 353 F10/11 lines was produced by crossing the japonica KBNT *lpa 1-1* mutant with the indica variety Zhe 733 [44]. This material, designated the K/Z mapping population, has been placed in the GSOR [43] collection at the DB NRRC, where it has become an often-requested population by geneticists and other scientists.

While in Arkansas the author also participated in an IAEA programme on Multi-location yield trials of rice mutants in 6 countries in the Latin American region: Brazil, Colombia, Costa Rica, Cuba, Guatemala and Uruguay. In this programme eight mutant lines were identified as potentially suitable for cultivation in cooperating countries and were incorporated into national rice trials [45]. In addition, desirable mutated characteristics were found which could be transferred through crossing programmes: earliness, salinity tolerance, blast resistance, resistance to shattering, translucent grain, high milling yield, plant height, and high yield potential

Another achievement in Arkansas was the development of *aromatic se* germplasm as a semidwarf(s), early maturing (e) recombinant from the cross between a late maturing semidwarf mutant, DM 107-4, and the early maturing tall variety Kashmir Basmati [46]. Both of the parents were induced mutants of Basmati 370, developed by M. A. Awan in Pakistan [25]. Although *aromatic se* retains the aroma and cooking quality of the original basmati source, yield has been low relative to conventional Arkansas varieties [46]. Efforts to develop additional aromatic varieties through induction and inter-crossing of mutants are underway.

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Induced Mutations in Plant Breeding and Biological Researches in Japan

H Nakagawa

Abstract

Two hundred and forty two direct-use mutant varieties generated by using irradiation, chemical mutagenesis and somaclonal variations, have been registered in Japan. About 61% of these were induced by Gamma-ray irradiation, largely due to successful collaboration with the Institute of Radiation Breeding. This high percentage of Gamma-ray irradiated mutants indicates that mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars. Some mutant cultivars of Japanese pear exhibiting resistance to diseases induced by Gamma-ray irradiation and development of a unique bioassay by using toxins of fungi was discussed. In addition, 228 indirect-use (hybrid) mutant varieties primarily generated in rice and soybean have found value as parental breeding germplasm resources in Japan. In 2005, two direct-use cultivars and 97 indirect-use cultivars of rice contributed approximately 12.4% of the total area of rice cultivation in Japan. The semi-dwarf gene (*sd-1*) generated in rice is perhaps one of the most significant contributions. For soybean, similar Gamma-ray induced mutants comprised nearly 9.4% of the total cultivation area of soybean in Japan. Molecular genetic studies focused on genome sequencing have become an extremely powerful tool for identifying the genes and for selecting mutants exhibiting specific phenotypes. It is anticipated that molecular genetic interaction will complement gains in mutation breeding on a dramatic scale. Chronic irradiation in the Gamma Field is also considered to be a useful tool for generating mutant resources for future molecular studies especially in rice, and expand its use into the other graminaceous crops which have genomic synteny to rice. There are interesting reports concerning mutations in rice, such as low glutelin content, in which the size and location of deletions and the mechanisms and phenotypes of low glutelin content were elucidated. Chronic irradiation in the Gamma Field is useful to generate mutant resources for molecular researches.

Introduction

After the construction of the Gamma Field, now considered the world's largest radiation field (Fig. 1, 100m radius with an 88.8 TBq ⁶⁰Co source at the center), the Gamma Room and the Gamma Greenhouse in the Institute of Radiation Breeding (IRB) in 1960's, mutation breeding was accelerated by cooperative research with national and prefectural breeding laboratories, private companies and universities in Japan [1].

In *The New York Times* (In "Useful Mutants, Bred With Radiation" by William J. Broad, August 27, 2007), Dr. P. J. L. Lagoda of the Joint FAO/IAEA was quoted to say, "Spontaneous mutations are the motor of evolution. We are mimicking nature in this. We're concentrating time and space for the breeder so he can do the job in his lifetime. We concentrate on how often mutants appear - going through 10,000 to one million - to select just the right one."



Figure 1 Gamma Field of IRB

The concept and objectives of the IRB's Gamma Field has the same goals for the plant breeder. The facility is used to artificially induce mutations at a higher frequency than it occurs in nature. The radiation dose at the nearest point of the field (10m from the center, ca. 2Gy/day) is estimated to be about 300,000 times that of normal and natural background radiation. At the farthest point (100m from the center, ca. 0.01Gy/day), the radiation dosage is about 2,000 times that of normal background radiation. This means that growing plants at the nearest point to the Gamma-ray sources are being treated to a 1,000 year's of accumulated normal background rates of radiation per day. Although we do not know all the genes or mechanism of mutations, radiation breeding has produced many useful mutant cultivars and contributed greatly to the farmers and industries of Japan.

In 1991, the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan launched the Rice Genome Research Programme (RGP), with the aim of fully decoding the rice genome in three phases over a 21-year period. With the cooperation of 10 participating countries [2], the genome sequencing of 12 rice chromosomes was completed in 2005 [3]. Following this achievement, molecular genetic studies based on the results of the genome sequencing project became the most powerful tool for selecting mutants of certain characteristics in rice. This is anticipated to revolutionize mutation breeding success in rice, and become applicable to a number of other important crop species.

In this report, the mutant cultivars developed mainly by Gamma-rays are discussed. In addition, their economic impacts in Japan, as well as molecular studies performed to elucidate the mutation at the DNA level are described.

Mutation breeding and released cultivars in Japan

In a 2007 search regarding the number of induced mutation varieties in the IAEA database, China is first in the number of described induced

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mutation varieties at 638; India is second listing 272 varieties; and Japan is third with 233 varieties. The total number of mutant cultivars, including direct-use mutant cultivars and indirect-use cultivars, exceed these totals. A selection of mutant cultivars developed in Japan, including the economic impact of these cultivars, and their characteristics are reviewed here.

The number of cultivars developed by mutation breeding

Figure 2 shows the number of direct-use and indirect-use (hybrid) mutant cultivars registered in Japan in each five-year period from 1960 to 2005. The number of direct-use cultivars had been rapidly increasing until 1995, when 67 cultivars were registered in five years (about 13 cultivars per year). This number fell from 2001 to 2005, with only 41 cultivars being registered (about 8 cultivars per year). The number of indirect-use cultivars primarily generated in rice has steadily increased over time and 68 cultivars were registered from 2001 to 2005. This number can be increased if agronomically useful, direct-use cultivars, such as “Reimei” with the *sd1* dwarf gene for rice are developed.

Two hundred and forty two direct-use mutant cultivars comprising 61 species generated through irradiation utilizing Gamma-ray, X-ray and ion beams, chemical mutagenesis and *in vitro* culture (somaclonal variation), have been registered and released in Japan (**Fig. 3**). More than 61% of these were induced by Gamma-ray irradiation and those induced by somaclonal variation and chemical mutagen, not including those with double chromosome numbers through colchicine treatment, are 15.7% and 6.6%, respectively. Recently, the development of mutant flower cultivars, generated by ion beam irradiation, has been a growing area of mutation induction in Japan.

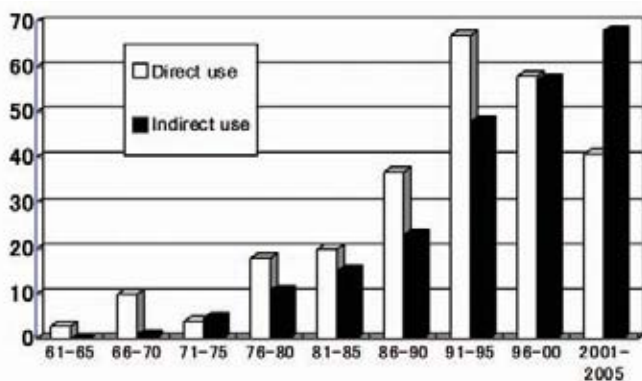


Figure 2 Number of cultivars developed by mutation breeding in each 5-year period from 1961-2005. Total number of direct use cultivars is 212 and that of indirect use cultivars is 230 [4].

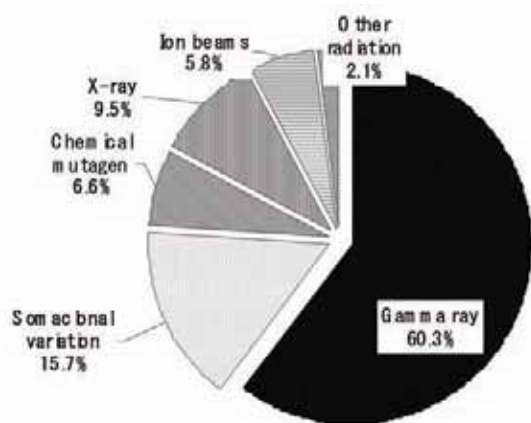


Figure 3 Percentage of total 242 cultivars developed by mutation breeding by using various kinds of methods in Japan (2008). Chemical mutagen does not include colchicine [4].

Table 1 shows the number of registered mutant cultivars of some crops developed by radiation, Gamma-rays, and those irradiated at the IRB, NIAS [4]. There are 50 mutant cultivars of chrysanthemum, 31 of rice, 16 of soybean, 10 of rose, etc. Among them, 100 cultivars have been generated at the IRB and these contributions of the IRB regarding the development and release of superior mutant induced cultivars has been extensive. This high percentage of Gamma-ray irradiated mutants indicates that mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars.

The first mutant rice cultivar is “Reimei,” which means “dawn” in Japanese, was the first irradiation induced mutant cultivar that illustrated the potential of utilizing Gamma-rays for breeding improvements in Japan. Reduction of plant height, including dwarfism and semi-dwarfism is one of the characteristics that can be induced with high frequency by irradiation and can be easily detected in the field. “Reimei,” registered in 1966 [5] was a successful case of an irradiation induced semi-dwarf mutant. This cultivar exhibits a mutation of the *sd-1* locus [6] and shows a culm 15cm shorter than the original cultivar “Fujiminori.” The semi-dwarf is associated with the high-yielding ability and recorded the highest yield in Japan in 1967 [5].

Table 1. Number of registered mutant cultivars developed by radiation, Gamma-rays, and those irradiated in the Institute of Radiation Breeding, NIAS [4]

	Mutant cultivars ¹	Radiation	Gamma-rays	IRB ²
61 Crops	242	188	146	100
Rice	31	14	12	11
Wheat	4	2	2	0
Barley	4	4	3	0
Soybean	16	16	15	9
Chrysanthemum	50	46	32	29
Rose	10	7	7	6
Sea pink (Limonium)	6	6	6	0
Cytisus	8	8	8	8
Apple	2	2	2	2
Japanese Pear	3	3	3	3
Others	108	80	56	32

¹ Total number of mutant cultivars developed by radiation (Gamma-ray, X-ray and ion beams), chemicals (Excluding colchicine treatment), somaclonal variation

² Number of mutant cultivars irradiated in the Institute of Radiation Breeding (IRB)

Table 2. Number of indirect use mutant cultivars in Japan (2008)

Rice	Wheat	Barley	Soybean	Tomato	Others	Total
198	3	7	9	3	7	228

In Japan, the total number of indirect-use mutant cultivars is 228, which includes 198 rice, 9 soybean, 7 barley, 3 wheat, 3 tomato, 4 lettuce, 1 eggplant, 1 Japanese lawngrass, 1 mat rush, and 1 mushroom cultivar in 2008 (**Table 2**). Interestingly, among the 198 indirect-use mutant cultivars in 2008, 89 cultivars (44.9%) were derived from the “Reimei” or its offspring. This suggests that agronomically useful mutations can be utilized as parental lines to develop new varieties with this characteristic and transferred efficiently to the farmers’ field.

The Economic impact of mutant cultivars in Japan

Figure 4 shows the increase of mutant rice cultivars, which were derived from mutants generated by Gamma-rays, planted in farmers’ fields in Japan since 1960. “Reimei” was first cultivated on 61,598 ha in 1968,

(<http://ineweb.narcc.affrc.go.jp/>). The number of mutant cultivars has been increasing and 99 mutant cultivars (2 direct-use and 97 indirect-use cultivars) were in cultivation in 2005 [4].

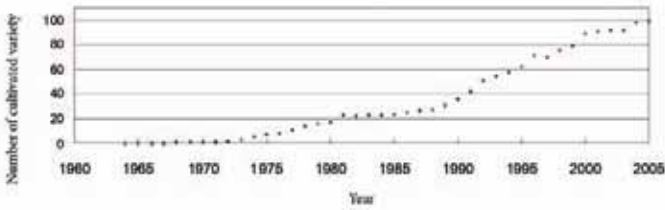


Figure 4 Total number of mutant rice cultivars, which are derived from mutants generated by Gamma-rays, cultivated in farmers' field from 1960 to 2005 in Japan [4].

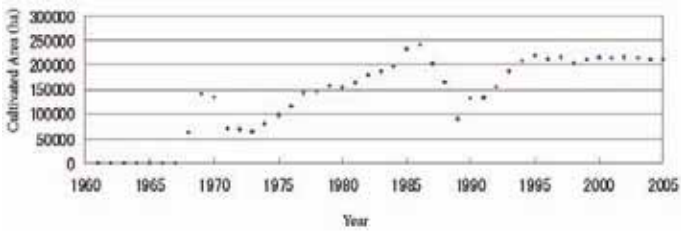


Figure 5 Total areas of mutant rice cultivars, which are derived from mutants generated by Gamma-rays, cultivated in farmers' field from 1960 to 2005 in Japan [4].

Figure 5 shows the total cultivated field of the mutant cultivars, which are derived from mutants generated by Gamma-rays, from 1961 to 2005. This increased after "Reimei" was released for cultivation in 1968. The peak use of mutant induced cultivars reached 250,000 ha in 1986 and was slightly more than 200,000 ha from 1994 to 2005. In 2005, the total cultivated area of mutant cultivars was 210,692 ha, which is 12.4% of total cultivated area of paddy rice (1,702,000 ha) in Japan [4].

The total crude income of farmers selling the brown rice of mutant cultivars also has been increasing as the increase of the cultivated area, although the price of the grain is different in each year. The amount of total income is estimated to be approximately 250 billion Yen (2.34 billion US dollars) in 2005 [4]. The mutant cultivars, which are derived from mutants generated by Gamma-rays and have been cultivated on more than 5,000 ha from 2001 to 2005, are the following 17 cultivars, "Kinuhikari (263,223ha)"; "Haenuki (219,734ha)"; "Tsugaru-roman (106,423ha)"; "Yume-akari (66,491ha)"; "Yume-tsukushi (58,893ha)"; "Aichi-no-kaori (53,697ha)"; "Asahi-no-yume (51,049ha)"; "Mutsuhomare (46,959 ha)"; "Dontokoi (17,008ha)"; "Yume-shizuku (14,076ha)"; "Mine-asahi (10,698 ha)"; "Yume-hitachi (10,440ha)"; "Yume-minori (9,957ha)"; "Aki-geshiki (7,510ha)"; "Aki-roman (7,450ha)"; "Miyama-nishiki (7,242 ha)"; and "Tsukushi-roman (5,533 ha)." The mutant cultivars, which have been cultivated in more than 100,000ha of farmers' fields are the following 5 cultivars, "Akihikari (1,410,810ha)"; "Reimei (886,188ha)"; "Kinuhikari (263,223ha)"; "Haenuki (219,734ha); and "Tsugaru-roman (106,423ha)." Among them, "Reimei" is a direct-use mutation cultivar and the others are indirect-use cultivars [4].

There are 16 direct-use mutant cultivars of soybean registered in Japan since "Raiden" and "Raikou" were developed by Gamma-ray irradiation in 1960. The improved characteristics were early-maturity and late-maturity, yellow hilum, seed-coat color, short-stem, and the number of pods/stem, lipoxygenase-free, low allergen etc. Among them, one cultivar was induced by X-ray and the other 15 were induced by Gamma-rays. The number of indirect-use cultivars is 10. The total cultivated area of mutant cultivars cultivated in the farmers' fields came to 13,283 ha (9.4% of total cultivated area (142,000ha) of soybean in Japan in 2005)

and total farmers' crude income was 5.56 billion Yen (ca. 52 million US dollars) [4]. As a result, economic impact of mutant cultivars is huge in Japan.

Some useful mutant varieties by using various screening methods

Rice

Although rice is not a high protein grain crop, the protein content is ca. 7% when the white rice is cooked. A mutant line with a low content of glutelin was obtained from the ethyleneimine (EI) treatment to "Nihonmasari." The "LGC-1" was developed from back-crossing this mutant with the original "Nihonmasari" to eliminate undesirable characteristics, such as semi-sterility and semi-dwarfism [7]. The seed protein of the "LGC-1" is composed of mainly of a low amount of digestible glutelin and high amount of indigestible prolamine. This construction of protein is disadvantageous for the digestion of rice grains in humans, though the total amount of protein is mostly similar to the original cultivar. As a result, the "LGC-1" is useful as "low protein rice," and some clinical trials on patients with kidney disease indicate that the variety is a useful and effective daily food for such patients [8]. The defect of the "LGC-1" is its eating quality, and there are the other loci that control the biosynthesis of digestible protein, such as globulin. Therefore, Nishimura, *et al.* [9] induced a mutant named "89WPKG30-433" with a deficiency in globulin from the leading Japanese cultivar "Koshihikari" through Gamma-ray irradiation. They hybridized it with the "LGC-1" and selected "LGC-Katsu" and "LGC-Jun" from the hybrids, whose globulin content was as low as the "LGC-1," where the globulin content is zero. The total digestible protein content tested to about 30% of ordinary rice. As the eating quality is highly improved and digestible protein content is lower than "LGC-1," these two cultivars will greatly help in the dietary management of proteins with chronic renal failure.

Soybean

Takagi [10] identified two major genes, which control radio-sensitivity, in some soybean varieties. When the 50% reduction rate (RD₅₀) of root length was determined with acute irradiation to the seeds or the chronic irradiation to the plants for the entire growth period, radio-sensitivity of a sensitive cultivar, "Shinmejiro," is more than twice that of the resistant variety, "Tachisuzunari." The differences in radio-sensitivity between the varieties to the chronic irradiation in the Gamma Field were controlled by a single recessive gene, *rs1*. Besides, the second recessive gene *rs2*, which was discovered in "Goishishirobana," whose activity is only expressed following acute seed radiation.

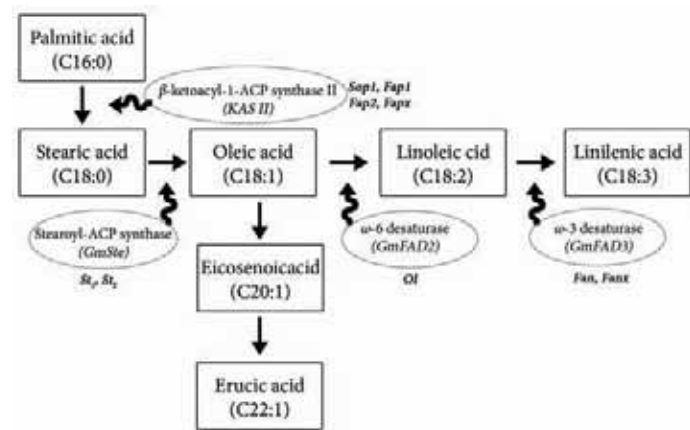


Figure 6 Metabolic pathway and key genes of fatty acid in soybean [13] (courtesy of Prof. Y. Takagi).

Soybean is the most widely used source of edible oil for human consumption. Fatty acids of soybean seeds consist of palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid (Fig. 6). Altered unsaturated fatty acid content (elevated oleic acid and reduced linolenic acid) increase the oxidative stability that provides health benefits and improvement of fatty acid contents. This has been one of the most important breeding objectives of soybean. As natural genetic diversity in soybean is limited, mutation induction is one effective approach to induce modification. Through the use of X-rays or chemical mutagens, mutants with different fatty acid compositions, such as reduced and elevated palmitic acid, elevated stearic acid, elevated oleic acid (50%), and reduced linolenic acid (3%) content were isolated and found to be controlled by major genes (Fig. 6; [11-13]).

Soybean seed has three lipoxygenases called *L-1*, *L-2* and *L-3*, respectively [14]. The lipoxygenases are the main factors of the grassy-beany flavor of the products. Soybean lines lacking each of the three genes have been developed. However, no line lacking all three genes has been obtained because of tight linkage between *L-1* and *L-2* [15]. The F_2 seeds derived from a cross between a line without *L-1*, *L-3* and a line without *L-2*, *L-3* were irradiated with Gamma-rays. After surveying 1,813 M3 seeds by using SDS/PAGE, one mutant seed lacking all *L-1*, *L-2*, and *L-3* was selected [16]. A new cultivar “Ichihime” with this unique characteristic was registered and released in 1994 [17].

Italian ryegrass

Mutation breeding has been mainly established in seed propagated, self-pollinated species. Although several methods have been widely used for the screening of mutants in self-pollinated species by the single-seed descent approach [18,19] and by single seed descent (one-plant-one-grain method, Yoshida [20]), these methods have not been applied to cross-pollinated species. Ukai [21] developed a new method for obtaining mutants of cross-pollinated species efficiently in a temperate forage grass, Italian ryegrass (*Lolium multiflorum* L.). The method was called the “Crossing-within-Spike-Progenies Method.” This method is composed of 1) taking seeds separately from each spike from a population of plants irradiated with Gamma-rays, 2) sowing the seeds in a hill plot as a spike-progeny, 3) isolating each hill from others at the time of flowering and allowing the open-pollination of plants within hills, and 4) taking seeds from each of the hills and sowing the seeds in hill progenies for the screening of mutants. This procedure is repeated each year. When 300Gy of Gamma-ray was irradiated to the seed, the frequency of chlorophyll mutations was approximately 70.6% per hill progeny and 1.87% per plant. In contrast, open-pollinated populations exhibited that only 10% per progeny and 0.12% per plant, respectively. This method will be applied to the other wind- or insect-pollinating outcrossing crop species.

Chrysanthemum

In general, it is very difficult to isolate mutants from mutation sectors in vegetatively propagated crops although the maintenance of mutant genotypes is easier than the seed-propagated species. It has been shown that the combined method of chronic Gamma-ray irradiation and tissue culture is very effective in solving this problem. By tissue culturing the floral organs of chrysanthemum (*Chrysanthemum morifolium* Ramat.) plants chronically irradiated in the Gamma Field from the seedling to the flowering stages, many non-chimeric mutants, with various flower colors and shapes, are obtained [22]. From these mutant lines, 10 cultivars were registered. The technology, given the term “radiobiotechnology,” is not only effective in obtaining non-chimeric mutants but also effective in producing high mutation frequencies. The method has been utilized to induce mutations in various vegetatively propagated crops and many mutant cultivars have been registered.

Japanese pear and apple resistant to *Alternaria* disease

A popular cultivar of Japanese pear (*Pyrus serotina* Rehd. var. *culta* Rehd.), “Nijisseiki,” which was a leading variety, occupied 28% of the total cultivated area of Japanese pear in 1990 in Japan. The cultivar, however, is highly susceptible to the black spot disease, *Alternaria alternata* (Fr.) Keissier (= *Alternaria kikuchiana* Tanaka), one of the most serious diseases of pear [23]. Growers are required to spray fungicides several times during the growing season to counter the disease. To induce mutations resistant to the disease by Gamma-ray irradiation, small plants of the cv. “Nijisseiki” were planted at every 4 meters from 37 m to 63 m from the ^{60}Co source in 1962 and chronic Gamma-ray irradiation was applied (30 x 10-2Gy - 4 x 10-2Gy /day) in the Gamma Field [24]. In 1981, nearly 20 years after the planting, a twig without the symptom of the disease was found in a plant planted at a distance of 53 m from the irradiation source. As it was ascertained that there was no difference in other agronomic characteristics between the mutant and the original variety except for the resistance to the black spot disease, it was registered and released in 1991 with the name “Gold Nijisseiki” [24]. It was registered as the same name in Australia in 2004 (Certificate Number 2533).

Dr. Sanada, one of the breeders of this cultivar, mentioned, “The situation of mutation breeding on fruit trees has been severely criticized because there have been no successful results.” Although it took them nearly 20 years to identify a useful mutation and 30 years for the registration, the release of “Gold Nijisseiki” is a monumental achievement for the Gamma Field.



Figure 7 Bioassay of resistant to the black spot disease by using the AK-toxin obtained from the culture of the fungus. Upper to lower leaf disc (1 – 5) means 1 (young) to 5 (older) leaf; cv. “Chojuuro”, highly resistant; cv. “Nijisseiki”, highly susceptible; cv. “Gold Nijisseiki”, resistant.

At the same time an easy and effective method for the screening of resistance to the fungus has been developed by treating leaf discs (7 mm in diameter) by the AK-toxin produced by the fungus [25]. It was coincidental and lucky for the breeders that Nakashima, *et al.* [26,27] isolated and identified the chemical structure of the toxin named “AK-toxin” produced by the fungus of black spot disease and generating the symptom of black spots on leaves at the same time. As a consequence, the breeding group entered into a cooperative research programme with the chemistry group and established this unique method. When the leaf discs are placed on the filter paper soaked with AK-toxin obtained either from the extract of the fungal body or artificial synthesis in a Petri dish, and kept for two or three days, susceptible leaves turned to black and resistant leaves stayed green (Fig. 7). After the development of this method, two new mutant varieties, “Osa-Gold [28,29]” and “Kotobuki Shinsui [30]”

were developed in a short period of time by using this screening method. The economic effect of this research has been great [4].

These researches suggest that the breeding of fruit trees requires patience and that development of easy and precise screening methods is a very important addition to the development of methods for mutation induction.

Achievement of biological researches on mutations induced by Gamma-ray irradiation

Deletion size generated by Gamma-ray

Naito, *et al.* [31] studied the deletion sizes of transmissible and non-transmissible mutations induced with Gamma-ray and carbon ion beam irradiation by the sophisticated pollen-irradiation methods in *Arabidopsis*. It has been revealed that most mutants induced with these ionizing irradiations possess extremely large deletions (more than 6 Mbp), most of which are not transmittable to the next generation, as well as small deletions (1 or 4 bp), which are normally transmissible.

In rice, the same tendency was observed in transmissible mutants. Morita (unpublished) researched the frequency of transmission of different mutations possessing different deletion sizes as obtained with Gamma-ray irradiation in rice. Among 11 Gamma-ray induced mutants, one *GluA2* mutant exhibited 1 base pair (bp) substitution, and among 10 mutants with a deletion, the deletion size of 6 mutants, which include *CAO* (*chlorophyllide-a oxygenase*), *GA3os* (*GA3-beta-hydroxylase*), *GluA1* (*glutelin A1*), and *GluA2* (*glutelin A2*) are 1 bp deletion, and those of the other *CAO* mutants and *PLA1* (*Plastochron1*) are 3 and 5 bp deletions, respectively. Those of *GluB4/5* (*Glutelin B4/5*), two α -globulin mutants are more than 10 kbp, 15 kbp, and 90 kbp, respectively. It is very interesting that the Gamma-ray induced mutations transmittable to the next generation are primarily classified into 2 groups, the one with extremely a large deletion and the other with small deletions (1 to 5 bp). We are not sure whether or not it is very difficult to obtain mutants with medium deletion size by Gamma-ray irradiations. However, we are accumulating data to elucidate it.

Different size and location of deletion generates different kinds of phenotypes

In the course of plant evolution, genes are often duplicated in tandem, resulting in a functional redundancy. The analysis of function of these genes by developing double mutants might be difficult because they would be very tightly linked. Mutants of such tandem duplicated genes were investigated for their genotypes and phenotypes. There are reversely repeated two loci, which both codes for mRNA of glutelin production. There are various mutants that exhibit low glutelin contents isolated by SDS-PAGE [7, 32]. The mechanisms of low glutelin contents of mutants that have been studied suggest that the size and the position of deletions generate different characteristics of mutations. Some act as dominant genes or recessive genes, and those relationships between genotypes and phenotypes, etc. are provided as example below.

Mechanism of low glutelin content in the “LGC-1” mutant

The *Low glutelin content* (*Lgc-1*) is a dominant mutation that reduces glutelin content in the rice grain. Glutelin is a major digestible seed storage protein encoded by a multigene family. Kusaba, *et al.* [33] reported that in *Lgc-1* homozygotes, there is a 3.5 kbp deletion between two highly similar glutelin genes that forms a tail-to-tail inverted repeat, that might produce a double stranded RNA molecule, a potent inducer of RNA silencing (Fig. 8). As a result, glutelin synthesis is suppressed and the glutelin content is lowered. The *Lgc-1* provides an interesting example of RNA silencing occurring among genes that exhibit various levels of similarity to an RNA-silencing-inducing gene. This was the first report that shows the mechanism of a mutation was RNAi.

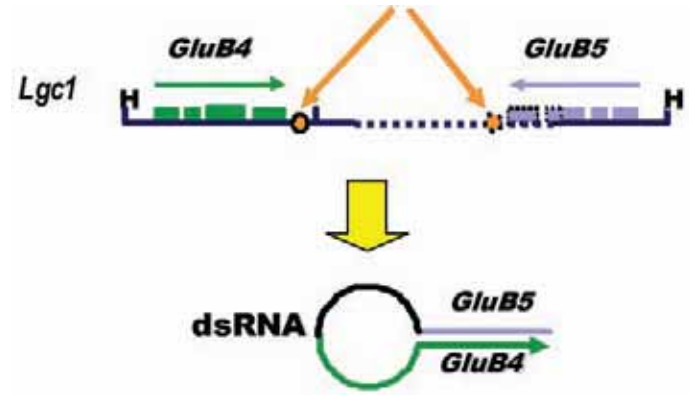


Figure 8 Mechanism of low glutelin in LGC-1 through a deletion at the transcription termination signal and produced double-stranded RNA suppress the glutelin synthesis by RNAi [33] (by courtesy of Prof. M. Kusaba, Hiroshima University).

Mechanism of low glutelin content in the “glu1” mutant

The “*glu1*” is a gamma-ray-induced rice mutant, which lacks an acidic subunit of glutelin, a major seed storage protein. Morita, *et al.* [34] elucidated that the *glu1* harbors a 129.7 kbp deletion involving two highly similar and tandem repeated glutelin genes, *GluB5* and *GluB4*. The deletion eliminated the entire *GluB5* and *GluB4* gene except half of the first exon of *GluB5*. As a result, the phenotype of the *glu1* gene is a complete lack of the acidic subunit of glutelin and acts as a recessive gene for low glutelin content in rice grains (Fig. 9).

Conclusion

The above examples illustrate that the position and the size of deletions in the same loci have the capacity to dramatically alter the phenotype of mutants through the process of transcription and translation. The *glu1*, which has a large 129.7 kbp deletion, acts as a recessive gene, while the *LGC1*, which has 3.5 kbp deletion including probably a terminal signal of the transcript region acts as a dominant gene.

Furthermore, the *GluB5* and the *GluB4* have the same amino acid sequence in their acidic subunit, suggesting that only the mutation involving both *GluB5* and *GluB4* result in the resultant phenotype. That is the lack of the glutelin acidic subunit deleted in the “*glu1*” mutant. It probably is very difficult to knock out both loci by chemical treatment or transposon techniques. Sequenced plant genomes exhibited more than 14% of the genes formed tandem array [3, 35]. This finding, however, suggests that Gamma-rays can be an effective mutagen to generate knock-out mutants of both loci and to analyze tandem repeated and functionally redundant genes.

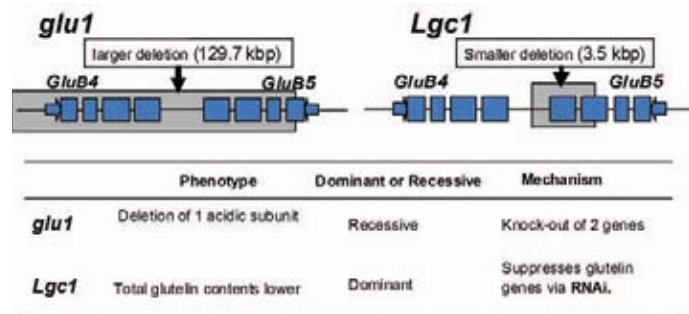


Figure 9 Comparison of phenotype, mode of inheritance and mechanism of mutation character between *glu1* and *Lgc1* mutation with different size and place of deletion in the same region of 2 loci, *GluB4* and *GluB5* (by courtesy of Dr. R. Morita, IRB, NIAS). *glu1*, Morita *et al.* [34] ; *Lgc1*, Kusaba *et al.* [33]

Genetic studies by the useful mutations induced with Gamma-ray chronic irradiation

As the history has shown, spontaneous and induced mutation resources have played an important role not only for mutation breeding but also genetic studies and the elucidation of gene functions.

Phytochrome

Takano, *et al.* [36] have isolated *phytochrome B* (*phyB*) and *phy C* mutants from rice and have produced all combinations of double mutants. Seedlings of *phy B* and *phyB phyC* mutants exhibited a partial loss of sensitivity to continuous red light but still showed significant deetiolation responses. The responses to red light were completely canceled in *phyA phyB* double mutants. These results indicate that *phyA* and *phyB* act in a highly redundant manner to control deetiolation under red light. They also found that mutations in either *phyB* or *phyC* locus causes moderate early flowering under a long-day photoperiod, while monogenic *phyA* mutations had little effect on flowering time. The *phyA* mutation, however, in combination with *phyB* or *phyC* mutation caused dramatic early flowering. Early flowering mutants were generated by chronic Gamma-ray irradiation with dose rates ranging between 3 and 6Gy/day [36].

Aluminum tolerance

Ma, *et al.* [37] isolated a mutant with highly sensitivity to aluminum concentration from cv. Koshihikari of japonica rice, which has an aluminum resistance [38]. The mutant was induced with chronic Gamma-ray irradiation and exhibited the same phenotype to the wild type with the absence of aluminum. That is, M₁ plants were irradiated in the Gamma Field from seven days before heading to two days after heading under 20Gy/day for eight days. The root elongation of the mutant, however, was highly inhibited in the presence of 10 µM Al. The mutant also exhibited poorer root growth in acid soil. Genetic analysis showed that the high sensitivity to Al is controlled by a single recessive gene. The gene was mapped to the long arm of chromosome 6.

Conclusion

The Gamma Phytotron was established in Korea in 2005 and the Gamma Greenhouse, approximately doubled the size of the Gamma Greenhouse located at the IRB, Japan, was established in Malaysia in 2008. Both facilities are focused on the induction of mutation by chronic Gamma-ray irradiation to growing plants of important crop species. As described earlier in this report, chronic irradiation is a useful tool for the generation of mutant genome resources that have application toward molecular analysis as well as conventional breeding.

Conclusions

A. M. van Harten [39] describes in “Mutation Breeding -Theory and practical application,”

“An explanation for the decreasing interest in mutation breeding, at least in most “developed” countries, may be that during the past two decades attention has become more and more directed towards studying the possibilities offered to plant breeding by various new molecular technologies...As a result of these developments mutation breeding seems to have lost part of its previous attraction for young researchers.”

It is not necessary to mention, however, that mutation breeding is still a very interesting and useful technology for isolating genes and for elucidating gene mechanisms and metabolic pathways in various crops.

The record has also shown that mutation induction is a very useful conventional breeding tool for developing superior cultivars. Today, site-directed mutagenesis *in vivo* or *in vitro* cell can be envisioned and many researchers are conducting programmes in this direction.

New fields of science and technologies were developed on the basis of achievements of traditional or classic methods. It is highly desirable that

the IRB continues their work while incorporating the new knowledge and technologies. The IRB is well equipped with appropriate facilities and equipment that will contribute to the future mutation breeding developments and be a contributor in solving the problems mentioned in this review.

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Mutation Breeding in Oilseeds and Grain Legumes in India: Accomplishments and Socio-Economic Impact

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Abstract

In India, oilseed and legume crops are important food components as they are major contributors to dietary oils and proteins. In order to generate genetic variability in these crops, mutation research is extensively carried out at Bhabha Atomic Research Centre (BARC), Mumbai for the past half a century. Besides cytogenetic studies, the era of direct mutants as crop varieties began in groundnut, mustard, pigeonpea and mungbean. Induction of modified traits and their incorporation in an ideal genotype was achieved by judicious use of induced mutation and hybridization techniques. So far, 110 varieties in oilseed and legumes have been developed in India by incorporating desirable traits like large seed, semi-dwarf habit, high harvest index, better partitioning, fresh seed dormancy, yellow seed color, drought tolerance, resistance to powdery mildew, yellow mosaic virus, and bacterial pustule diseases. Many of the national/state breeding programmes have been utilizing these varieties as parental materials/donors and developed several improved varieties. Several of these varieties have high patronage from the farming community and are extensively grown in the country. Groundnut varieties have made considerable impact by giving record yields across the country. Further, mungbean varieties were also surging ahead by virtue of their resistance to yellow mosaic virus, *Rhizoctonia* root-rot and powdery mildew diseases with suitability to rice fallow situations. Blackgram variety TAU-1 has occupied maximum blackgram area in Maharashtra state. These varieties also facilitated farmers to develop newer cropping systems. Thus, induced mutation research remained in the forefront of Indian agricultural research by developing popular varieties with higher productivity potential in oilseeds and legumes.

Introduction

India produces variety of oilseeds and grain legumes, which constitute around 12% and 7% of the total food grain production, respectively [1]. Large proportion of Indian population relies on grain legumes as a dietary source of proteins due to economic or cultural reasons. India is the fifth largest oilseed producer accounting for 8% of the global oilseed production. Soybean contributes 31.4%, rapeseed/mustard 28.2%, groundnut 27.7% and sunflower 4.8% to Indian oilseed production [1, 2]. Among the food legumes, chickpea is the major Indian legume contributing 40.2% to country's total legume production followed by pigeonpea (17.4%), blackgram (10.6%) and mungbean (8.6%) [1]. National productivity levels compare poorly with rest of the world. In 2006, India imported 4.17 million tonnes of edible oils and 1.61 million tonnes of legumes worth of 2.57 billion USD [1]. Annual consumption of these is expected to increase further with increased urbanization, higher disposable incomes and burgeoning population, necessitating more imports. Many of the issues attributed to lower productivity were narrow genetic base, lack of tolerance to biotic and abiotic stresses, lack

of quality seeds of improved varieties and restriction of these crops on marginal areas with poor inputs.

Development of mutant varieties in oilseeds and grain legumes

Mutation breeding has played a significant role in the last 75 years by releasing around 2,672 mutant varieties for commercial cultivation in the world [3]. The major contribution is from cereals followed by ornamentals, legumes and oilseeds. Most of mutant varieties were released in China (27.7%), India (12.7%), Russia (9.8%), Japan (8.7%), Germany (6.5%), Netherlands (6.5%), USA (4.7%) and others (22.9%). Many induced mutants were released directly as new varieties, others used as parents to derive new varieties. Nearly 400 mutant varieties have been released in oilseeds and legumes in the world, of these 110 varieties were released from India.

In India, mutation breeding is being carried out in several national/state universities/institutes like Bhabha Atomic Research Centre (BARC), Indian Agricultural Research Institute (IARI), National Botanical Research Institute (NBRI), Tamil Nadu, Agricultural University (TNAU), etc. Under the peaceful uses of atomic energy in agriculture, BARC had initiated radiation based mutation techniques for the genetic enhancement of oilseeds and legumes more than five decades back. At present, BARC has been concentrating on major oilseeds of country's interest like groundnut, mustard, soybean, sunflower and legumes such as pigeonpea, mungbean, blackgram and cowpea [4]. Initial research activities focused on the effect of radiation on oilseeds and legumes, induction of wide spectrum of mutants for various traits, and genetic and cyto-genetical studies of mutant traits. In most of the mutation experiments, the objectives were to develop high-yielding varieties with early maturity, large seed, high oil content, moderate seed dormancy, ideal ideotype, tolerance to biotic and abiotic stresses and improved seed quality traits. Both chemical and physical mutagens were used for induced mutagenesis in oilseeds and legumes. Initial germplasm used for mutagen treatment was seeds of cultivar, mutant, selection, hybrids or advanced lines. Induced mutants are utilized directly for varietal development or in recombination breeding by hybridizing mutant X mutant, mutant X cultivar, mutant derivative X mutant or mutant derivative X cultivar. Varietal development using mutation with recombination breeding in oilseeds and grain legumes and their accomplishments and societal impact is briefly discussed here.

Sustained induced mutagenesis in oilseeds and grain legumes using X-rays, beta rays, Gamma-rays, fast neutrons, ethyl methane sulphonate and sodium azide resulted in wide spectrum of mutants affecting various traits (Table 1). Of these, six mutants of groundnut, three of sesame and one of sunflower were registered with National Bureau of Plant Genetic Resources (NBPGR), New Delhi for their mutant traits. First groundnut mutant, TG 1 with superior agronomic performance was released for commercial cultivation in 1973 [5]. Later research efforts with the initial X-ray irradiation followed by intermittent gamma irradiation and cross breeding exploited interaction of mutant X mutant, mutant X cultivar, mutant X breeding line genomes in turn resulted in wide spectrum of

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genetically diverse, agronomically important breeding lines. Planned irradiation had broken undesirable linkages and enhanced favorable recombinants. As a result, traits like large seed, increased harvest index, assimilate partitioning, semi-dwarf habit, earliness, new ideotypes, improved seed quality, and enhanced disease resistance were incorporated in oilseed and legumes.

The effective blend of mutation and recombination breeding at our and other institutes resulted in the release of 50 oilseeds and 60 legume varieties for commercial cultivation in the country (Table 2). Among these, 33 varieties have been released by BARC [4]. These varieties were evolved by incorporating desirable agronomic features like large seed in TG 1, TKG 19A, Somnath (TGS 1), TPG 41 and TLG 45 (groundnut); TAT 10

and TT 6 (pigeonpea); TAP 7, TM 96-2 and TMB 37 (mungbean); TAU 2 (urdbean); semi dwarf habit, high harvest index and better partitioning in TAG 24 (groundnut), TRC 77-4 (cowpea); fresh seed dormancy in TG 22 and TG 26 and drought tolerance in TG 37A (groundnut). Additionally, powdery mildew resistance in TARM 1, TARM 2, TARM 18, TM 96-2; powdery mildew and yellow mosaic resistance in TMB 37 and TJM 3 (mungbean), bacterial pustule resistance in TAMS 38 and multiple pest resistance in TAMS 98-21 (soybean); yellow mosaic virus resistance in TU 94-2 (urdbean) were also introduced in these varieties. Mutant varieties like Aruna of castor, Pusa 408 (Ajay), Pusa-413 (Atul), Pusa-417 (Girnar) of chickpea, Co-4, MaruMoth-1 of mothbean are among the important varieties of economic significance released in India.

Table 1. Spectrum of mutants in oilseeds and grain legumes maintained at BARC, Mumbai, India

Crop	Botanical name	No of mutants	Traits mutated	Mutagen used
Groundnut	<i>Arachis hypogaea</i>	176	Plant height, leaf, pod, seed, disease, oil, salinity tolerance	X rays, gamma rays, EMS, Sodium azide
Soybean	<i>Glycine max</i>	55	Plant height, leaf, protein traits, fatty acid, root nodulation, flower colour, trypsin inhibitor	Gamma rays
Mustard	<i>Brassica juncea</i>	12	Earliness, seed colour, low erucic acid, high oil, leaf type and colour, appressed pods, powdery mildew tolerance	Beta rays, gamma rays
Sunflower	<i>Helianthus annuus</i>	10	Plant height, leaf colour, seed colour, male sterility, high oil, less ray florets, small ray florets	Gamma rays
Mungbean	<i>Vigna radiata</i>	124	Plant height, leaf, branches, flowering, pod, seed, yield, disease resistance, drought tolerance, resistance to pre-harvest sprouting.	X rays, gamma rays, EMS
Blackgram	<i>Vigna mungo</i>	74	Plant height, leaf, branches, flowering, pod, seed, yield, pod shattering resistance, disease resistance	X rays, gamma rays, EMS, Sodium azide.
Pigeonpea	<i>Cajanus cajan</i>	25	Plant height, leaf, branches, flowering, pod, seed, yield, disease resistance	Gamma rays, fast neutrons, EMS
Cowpea	<i>Vigna unguiculata</i>	34	Plant height, leaf, branches, flowering, pod, seed, yield, disease resistance, drought tolerance	X rays, gamma rays, EMS, Sodium azide.

Table 2. Mutant varieties of oilseeds and grain legumes released for commercial cultivation in India

Crop	Botanical name	No of varieties	Mutagen	Traits improved
Groundnut	<i>Arachis hypogaea</i>	28	X-rays, Gamma rays, Sodium azide.	Large seed, early maturity, seed dormancy, high shelling out-turn, high harvest index, drought tolerance
Soybean	<i>Glycine max</i>	7	Gamma rays	Dwarf, earliness, bacterial leaf pustule resistance
Mustard	<i>Brassica juncea</i>	7	X-rays, beta rays, gamma rays	Earliness, large seed, high oil, seed coat colour
Castor	<i>Ricinus communis</i>	4	Fast neutrons, Gamma rays	Early, high yield, drought tolerance
Sesame	<i>Sesamum indicum</i>	3	Gamma rays, EMS	Dwarf, high yield, Cercospora leaf spot (CLS) tolerance
Sunflower	<i>Helianthus annuus</i>	1	Gamma rays	Seed coat colour
Mungbean				
Cowpea	<i>Vigna unguiculata</i>	8	Gamma rays, DMS	Earliness, green fodder, high yield, fodder
Chickpea	<i>Cicer arietinum</i>	7	Gamma rays, Fast neutrons	High yield, profuse branching, Fusarium wilt resistance, robust plant type, salinity tolerance
Blackgram	<i>Vigna mungo</i>	7	Gamma rays, X-rays	Large seed, terminal podding, tolerance to PM and YMV
Pigeonpea	<i>Cajanus cajan</i>	6	X-rays, Fast neutrons, Gamma rays, EMS	High yield, large seed, profuse branching, wilt resistance
Mothbean	<i>Vigna aconitifolia</i>	5	Gamma rays, EMS	High yield, YMV resistance, high protein, high harvest index
Lentil	<i>Lens culinaris</i>	3	Gamma rays	Large seed, protein content
Field bean	<i>Dolichos lablab</i>	2	Gamma rays	Large seed, Photo-insensitiveness
Pea	<i>Pisum sativum</i>	1	E I	Semi-erect, high yield
French bean	<i>Phaseolus vulgaris</i>	1	X-rays	Earliness, YMV resistance
Others		3	Gamma rays	High yield, earliness, large seed

Socio-economic impact of mutant varieties

The majority of the mutant varieties not only benefited the Indian farming community, but are also being used as genetic resource material in national/state breeding programmes. Among the groundnut varieties, TAG 24, TG 26, TG 37A in normal seed class and TKG 19A and TPG 41 in large seed class, became popular among the farming community in India. These are being used as check varieties in the respective national and state varietal trials. As a first step to transfer the benefits of these varieties to the farmers, large-scale breeder seed production programmes were undertaken

by involving several national institutes and state agricultural universities. In the last decade (1998-2008) 1,022 metric tons of breeder seed of these groundnut varieties worth 1.18 million US dollars was produced and supplied to various National and State Seed Corporations, State Agricultural Universities, seed companies, non-governmental organizations, farmers, etc. Based on the feedback received from National Seed Corporation, Pune, they supplied 1,190 metric tons worth 1.3 million US dollars of certified seed of TG varieties to farmers in the last five years. Further, several millions worth trading of groundnut mutant varieties has been taking place in most of the groundnut markets.

Farmers have been realizing the high-yielding ability of groundnut varieties by harvesting record groundnut yields in many parts of the country. By cultivating these mutant varieties, the groundnut productivity in major groundnut states like, Gujarat, Andhra Pradesh, Maharashtra, Karnataka, Orissa and Rajasthan has been almost doubled. Hundreds of farmers were harvesting significantly improved productivity even up to seven tonnes/ha and earning a net profit up to 1,200 US dollars/ha, when recently released groundnut varieties were introduced in these states [6]. Progressive farmers had harvested a record yield of more than 10 tonnes/ha dry pods in TAG 24 and TG 26 varieties by growing them under suitable agro-ecology such as summer environment, balanced nutrition and uninterrupted but controlled irrigation to achieve record yields in groundnut [7]. TAG 24 and TG 26 comprised most of the ideal morpho-physiological traits defined for groundnut. Both the varieties were grown at Field Research Laboratory, Leh at an altitude of 3,505 meters above mean sea level using polythene mulch. This might be the world's first report of groundnut cultivation at that altitude. According to Valls, *et al.* [8], some of the wild *Arachis* species are grown at an altitude of almost 1,600 meters. A drought tolerant variety, TG 37A has rekindled groundnut cultivation in desert areas of Rajasthan state. Existing large seed varieties were with long duration, longer seed dormancy and low productivity. However the recent release of large seed mutant varieties like TPG 41 and TLG 45 benefited many farmers, traders and exporters by virtue of their earliness, moderate seed dormancy and superior productivity.

Looking into advantages of these varieties, several organizations were conducting large-scale field demonstrations and many seed companies have taken up large-scale seed multiplication in order to reach larger pockets of farming community. Directorate of Oilseeds Development, Hyderabad had allocated 9,700 minikits of TG 37A and TPG 41 in major groundnut growing states. Trombay varieties also facilitated farmers to develop newer cropping systems like intercropping groundnut with sweet corn, Bt cotton, sugarcane; polythene mulch technology in groundnut. Our own estimates, breeder seed demand and feedback received from various agencies indicate Trombay groundnut varieties cover around 25% area of groundnut in the country.

In mungbean, BARC has made major break through in developing disease resistant varieties. TARM 1, TARM 2 and TARM 18 were the varieties released for the first time with powdery mildew resistance for southern and central zones of India [9]. TMB-37 has been released for summer season, having early maturity (55-59 days) and yellow mosaic disease resistance that made available an additional area for mungbean cultivation under crop diversity programme. Another mungbean variety TJM 3 is the first variety with multiple disease resistance for powdery mildew, yellow mosaic virus and *Rhizoctonia* root-rot diseases. The recently released mungbean variety TM 96-2 is the first variety for rice fallow cultivation in India, which has powdery mildew resistance and synchronous maturity which are the essential traits for rice fallow cultivation, where nearly four million hectares of rice fallow area is available in India and considered prime area under crop diversity programme. Disease resistance in Trombay mungbean varieties not only enhanced the productivity but also benefited in recovering quality seeds. Based on the breeder production during last few years, Trombay mungbean

varieties are estimated to cover around 10% (300,000ha) of mungbean area in the country.

Urdbean mutant varieties TAU 1 and TU 94-2 are very popular varieties in central and southern zones of India. TAU 1 ranks first for breeder seed demand every year. Based on the feedback received from Maharashtra State Seed Corporation, Akola and National Seed Corporation, Pune, they supplied 21,013 metric tons worth 18.5 million US dollars of certified seed of TAU 1 to farmers. Recently released soybean varieties, TAMS 38 and TAMS 98-21 are becoming popular among the farmers in Vidarbha region of Maharashtra state and are being cultivated on around 150,000 hectares [10].

Conclusion

Our experiences have shown that induced mutation has come to stay as an efficient plant breeding method towards improvement of oilseeds and legumes and development of commercial varieties for our farming community. Evidently, this methodology has benefited the farmers, traders, exporters and end-users and will continue to play a significant role in addressing food and nutritional security. In the present genomic era, induced mutants would be ideal genetic material for future functional genomic studies.

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Achievements of Grain Legume Variety Improvement Using Induced Mutation of the IAEA/RAS/5/040 Project in Thailand

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Abstract

IAEA/RAS/5/040 project aims to form a regional cooperation network of mutation germplasm with emphasis on seed-propagated crops among the Member States in Asia and Pacific commencing in 2002 and ending in 2006. It comprised of two components, the establishment and implementation of mutant multi-location trials and the establishment of mutation germplasm network. Thailand participated with two major grain legume crops, soybean and mungbean, of both components. Significant achievements are summarized. **Soybean mutant multi-location trials:** Two introduced mutants, DT84 from Vietnam and Bangsakong from Korea were well adapted in the upper and lower north of Thailand. DT84 produced similar yield and matured 18 days earlier than Chiang Mai 60 whereas Bangsakong gave 11% greater yield with 12 days earlier than Sukhothai. **Soybean mutants resistant to Soybean Crinkle Leaf:** The disease, caused by virus, is a major disease in Thailand. It is transmitted by whitefly (*Bemisia tabaci*). Seed of a soybean line cm9238-54-1(ST) was irradiated with 200 gray. A number of mutant lines were selected under natural field infections and tested in laboratory. Six mutant lines resistant to the disease were finally selected. **Soybean mutants with high grain protein:** The government policy is to increase grain protein soybean for soy food products. Seeds of three soybean varieties namely Chiang Mai 60, SSRSN19-35-4 and EHP275 were irradiated with 200 gray. Pedigree method of selection was used and grain protein of the mutants was analysed. Thirty two mutant lines were selected. The result of a preliminary trial showed that the lines gave average grain protein of 0.8, 2.0 and 1.0% higher than the original parents of 41.8, 40.3 and 41.9%, respectively. **Soybean mutants with high seed germination and vigor:** Chiang Mai 60, a high yielding soybean variety, has a poor character of seed germination and vigor. Seed of Chiang Mai 60 was irradiated with 100 gray. Pedigree method of selection was used in late generations. Accelerated Aging Test was also used to test the seed vigour of the mutant lines. In dry season trial, eight mutant lines had seed germination of 65-75% compared with the parent of 30%. In rainy season, 12 mutant lines had seed germination of 75-89% whereas the parent had only 41%. **Mungbean mutant multi-location trials:** The highest yielding variety across five trials during 2003-2005 was a Thai mutant, Chai Nat 72. It produced large seed of 70g/ 1,000 seeds which is a desirable trait for Thai and international markets. However, this mutant is susceptible to powdery mildew disease. An introduction from the Philippines, LM19-Native Variety, showed resistant to the disease. It can be utilized for mungbean breeding programme. **Novel mungbean germplasm derived from induced mutation; variegated leaf:** All F₁ plants from the cross between variegated mutant and normal leaf parent showed normal green leaves without reciprocal while the F₂ plants segregated well in a 3 : 1 ratio. The number of F₃ lines showing all green plants, segregating, all variegated plants fitted well with the 1 : 2 : 1 ratio. The variegated

leaf character is controlled by a single recessive gene. **Multiple leaflet:** A mutant with small pentafoolate was crossed with a large heptafoolate mutant to study the inheritance. It was found that their F₂ plants segregated in the ratio of 9,3,4 with tri (N_1-N_2-), penta ($N1-n2n2$) and heptafoolate ($n_1n_1N_2-$ and $n_1n_1n_2n_2$). The n_2 may be closely linked to the gene controlling leaf size as well. There are three AFLP markers linked to number of leaflets per leaf and all of them corresponded to the n_1 locus. The mutants of soybean and mungbean will be utilized for further breeding programme and demonstrated in farmers' field.

Introduction

IAEA/RAS/5/040 project, Enhancement of Genetic Diversity in Food, Pulses and Oil Crops and Establishment of Mutant Germplasm Network, aims to form a regional cooperation network of mutation germplasm with emphasis on seed-propagated crops among the Member States in Asia and Pacific. The project was first approved in 2002 and ended in 2006. It comprised of two components, the establishment and implementation of mutant multi-location trials and the establishment of mutation germplasm network. Thailand participated in two major grain legume crops, soybean (*Glycine max* L. Merrill) and mungbean (*Vigna radiata* L. Wilczek), of both components. Soybean is the most important grain legume in Thailand. The annual planted area during the past five years of 2002-2006 was 156,000ha with an annual production of about 232,000t [1], only 15% of the country's demand. Presently, 70% of the domestic demand is used for vegetable oil extraction and 10% is used for food products. Thai government policy is to increase the national productivity and improve grain quality of high protein for domestic consumption and for exports of soy food products. Mungbean is the second most important grain legume, occupying an annual planted area about 210,000ha during 2002-2006 with an annual production of 151,000t [1]. It can be cultivated three seasons of the year, the late rainy season commencing between late August and September is strongly recommended. Most of the annual mungbean production is used for bean sprouts. Therefore, germination and sprout quality are very important. Then bold seed is a preferred trait for domestic use and exports. Significant achievements of the research project are summarized.

Soybean mutant multi-location trials

The objective of this study was to evaluate grain yield and adaptability of introduced soybean mutants and their parents. The experiment was conducted at Chiang Mai Field Crops Research Center (FCRC) (18°14'N lat., 99°30'E lon., 316 m alt.) and Sukhothai Plant Production and Technical Service Center (PPTSC) (17°10'N lat., 99°52'E lon., 54 m alt.), the representative areas of the main soybean production in northern Thailand. RCB design was used with three replicates. A total number of 15 varieties included three mutants from Indonesia (GH-7, M-220, I-209), one parent and two mutants from Korea (Kumkangdaerip, KEX-2, Bangsakong), two mutants from Vietnam (M103, DT84), two parents and two mutants from China (HC18, HC18M, AJMD, AJMDM), and a parent (Chiang Mai 60), a mutant (CM60-10KR-71-PS-21) and a

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local check (Sukhothai 2) from Thailand. Plant population density was about 30-40 plants/m² with 50cm between rows, 20cm between hills and three to four plants per hill. Seed was inoculated with *Bradyrhizobium* at Chiang Mai FCRC. Fertilizer grade of 12-24-12 at a rate of 156 kg/ha was applied during 15-25 days after sowing (DAS). Pre-emergence herbicide was sprayed plus hand weeding prior to flowering. Insecticides were sprayed to control key insect pests, beanfly, whitefly, pod borers and bugs. Soybean was planted during either rainy or dry seasons. The amounts of rainfall during the crop duration in rainy season at Chiang Mai FCRC were 488 mm, 649 mm and 1,007 mm in 2003, 2004 and 2005 and at Sukhothai PPTSC were 546 mm and 672 mm in 2004 and 2005, respectively. In dry season 2004 and 2005, the plots were irrigated by flood-furrow system five times at Chiang Mai FCRC. In dry season 2005 at Sukhothai PPTSC, the plots were sprinkled six times with a total amount of 300 mm of water. Plants were harvested at 95% of pods reach their maturity.

Table 1. Soybean traits of exotic mutants compared with Thai varieties

Area/ variety	Grain yield (t/ha)	Days to maturity ²	100 seed wt. (g)
Upper north (5 trials, 2003-2005)			
DT84	1.74	83	15.2
Chiang Mai 60 ¹	1.69	101	13.7
Lower north (2 trials, 2005)			
Bangsakong	2.08	82	15.0
Sukhothai 2 ¹	1.89	94	14.5

¹ Thai check varieties
² days after sowing

At Chiang Mai FCRC, Chiang Mai 60, the most popular variety among Thai farmers in the upper north and central region, gave an average grain yield of 1.69 t/ha (Table 1), compared to the national average yield of 1.53 t/ha during 2003-2005 [1]. In general, the later days to maturity of the soybean, the greater the yield produced [2]. A mutant DT84 from Vietnam matured 18 days earlier than that of Chiang Mai 60, 101 DAS. However, it gave average grain yield of 1.74 t/ha, similar to that of Chiang Mai 60 (Table 1). In addition, DT84 had 11% larger seed size than Chiang Mai 60, 13.7 g/100 seeds. Desirable soybean grain of the Thai market for food products should have similar or larger seed size than Chiang Mai 60. DT84 is suitable to be grown in the rice-based cropping system and it is very promising for farmers in the upper northern area of the country [3].

At Sukhothai PPTSC, no introduction gave better yield than Thai variety Sukhothai 2, except a mutant Bangsakong from Korea produced the highest grain yield of 2.08 t/ha averaged from the 2005 trials, 11% higher than that of Sukhothai 2 (Table 1). Bangsakong matured 12 days earlier than Sukhothai 2, 94 DAS (Table 1). It produced similar seed size with Sukhothai 2, 14.5 g/100 seeds. Bangsakong is also a promising mutant for farmers grown in rice-based cropping system in the lower north of Thailand [3].

During 2006-2007, mutant varieties DT84 and Bangsakong were improved by the method of mass selection at Chiang Mai FCRC. The seed of two varieties was also multiplied for further test of their performance in farmers' fields prior to released.

Soybean mutants resistant to Soybean Crinkle Leaf

Soybean Crinkle Leaf (SCL) disease, caused by a virus, is a major disease in Thailand. It was first found in Thailand in 1979 [4]. It is transmitted by whitefly (*Bemisia tabaci*). Since 1998, the disease has been found in most main soybean-producing areas, in all growing seasons, and currently is a major constraint to soybean production [5]. Grain yield of soybean

can not be produced if the disease infects at the early stage of growth to flowering. Several insecticides are recommended to control whitefly. However, it is costly and not environmentally friendly. cm9238-54-1(ST) is a soybean promising line, it gave 5-10% higher grain yield than cv Chiang Mai 60. But the line is susceptible to SCL disease. Then seed of the line cm9238-54-1(ST) was irradiated with 200 gray. Pedigree method of selection was used from M₂-M₄. A number of mutant lines were selected under natural field infections at Lop Buri PPTSC and tested in laboratory at the Department of Agriculture, Bangkok. In laboratory, virus-free whitefly was transferred to infected soybean plants for a day. Then the disease agent was transmitted by releasing 30-40 whiteflies per plant for two days on M₄ seedlings lines. Thirty plants were used per line. The plants showing SCL symptom were observed at 40 days after transmission. Yield trials were conducted in 2005 and 2006 at Lop Buri PPTSC. In the 2005 trials, no insecticide was sprayed from emergence to flowering for free whitefly infestations. Later it was sprayed three times after flowering till full seed development to control pod borers and bugs. In the 2006 trial, insecticide was sprayed nine times from emergence to full seed filling stage to completely control whitefly and other insect pests.

Six mutant lines resistant to the disease were finally selected. The disease reaction under the laboratory test is shown in Table 2 compared with the original parent and two check cultivars. The results from the 2005 trials, the six lines gave average grain yield of 2.24-2.33 t/ha, 74-81% higher than that of the original parent under natural disease infections (Table 2). The yield reduction of the parent and check cultivars was due to the susceptibility to the disease leading to a decrease in the number of seeds per pod. Under no SCL disease symptom in the 2006 trial, the original parent gave the highest yield of 3.76 t/ha resulted from the highest number of pods per plant. However, the six mutants produced higher grain yield than the check cultivars. The mutant lines are being tested for their adaptability in farmers' fields.

Table 2. Grain yield and disease reaction of soybean mutant lines resistant to SCL disease in 2005 and grain yield under no disease symptom in 2006, Lop Buri PPTSC, Thailand

Mutant line/variety	Grain yield (t/ha)			SCL disease reaction
	dry season 2005	rainy season 2005	mean	
1-4	1.95 abc	2.54 ab	2.24	3.32 b R
16-42	1.81 bc	2.66 a	2.24	3.28 b MR
18-46	2.06 ab	2.59 a	2.33	2.80 c R
19-49	2.02 ab	2.58 a	2.30	3.38 b MR
20-50	2.14 a	2.36 b	2.25	3.26 b R
30-1	2.04 ab	2.61 a	2.32	3.31 b R
CM9238-54-1(ST)	1.76 c	0.82 d	1.29	3.76 a S
Chiang Mai 6 ¹	0.52 d	0.92 d	0.72	2.24 d VS
Sukhothai ¹	0.64 c	1.21 c	0.92	2.49 d S
Mean	1.66	2.03	1.84	3.09 -
CV (%)	7.6	8.1	7.9	6.0 -
Sowing dates	10 Jan	29 Jul	-	6 Jul -

Means followed by a common letter are not significantly different at 5% level by DMRT. Grain yield at 12% moisture, Season 2005 x Variety interaction is significant. Laboratory test of SCL disease, R = resistant, all plants showed no symptom; MR = moderately resistant, 1-10% of plants showed symptom; S = susceptible, 11-50% of plants showed symptom; VS = very susceptible, >50% of plants showed symptom. ¹ = check varieties.

Soybean mutants with high grain protein

The most popular soybean variety of Thai farmers is Chiang Mai 60. It gives about 36-38% of grain protein depending on locations and seasons.

The government policy is to increase grain protein of soybean for soy food products. Then soybean variety improvement to increase grain protein content was initiated. The seed of three soybean varieties namely Chiang Mai 60, SSRSN19-35-4 and EHP275 were irradiated with 200 gray. Pedigree method of selection was used at Nakhon Ratchasima FCRC and grain protein of the mutants was analyzed in laboratory at the Department of Agriculture, Bangkok [6].

A number of 32 mutant lines were selected. The result of a preliminary trial at Nakhon Ratchasima FCRC in 2007 showed that the lines gave average grain protein of 0.8, 2.0 and 1.0% higher than the original parents of 41.8, 40.3 and 41.9%, respectively [7]. Then it is possible to enhance grain protein percentage with similar or higher yield in the soybean varieties using induced mutation. The promising mutant lines will be further tested for their protein yield in farmer fields.

Soybean mutants with high seed germination and vigor

Chiang Mai 60 is a high yielding soybean variety in Thailand. However, it has a poor character of seed germination and vigor. Then induced mutation was used to improve seed quality of the variety, seed of Chiang Mai 60 was irradiated with 100 gray. Pedigree method of selection was used in late generations of M_4 - M_6 at Chiang Mai FCRC. Accelerated Aging Test was also used to test the seed vigor of the mutant lines.

A total number of 23 mutant lines were finally selected. In preliminary trials conducted in dry season 2006, eight mutant lines had seed germination of 65-80% compared with the original parent of 30%, whereas in rainy season 2006, 12 mutant lines had seed germination of 75-89%, and the parent gave only 41% [8]. The selected mutant lines are being tested for their yield in standard trials.

Mungbean mutant multi-location trials

The objective of this study was to evaluate grain yield and adaptability of introduced mungbean mutants and their parents. A total number of 18 mungbean accessions including introduced mutants, their parents and three Thai check varieties were tested for their yield and adaptability in the central region of the Kingdom, Chai Nat FCRC (15°15'N lat., 100°15'E lon., 16m alt.) and Kasetsart University, Kamphaeng Saen Campus (14°01'N lat., 99°58'E lon., 5 m alt.). Details of experiment carried out are shown in [9].

The highest yielding variety across five trials during 2003-2005 was a Thai mutant, Chai Nat 72. It produced large seed of 70 g per 1,000 seeds which is a desirable trait for Thai and international markets. However, this mutant is susceptible to powdery mildew disease. An introduction from the Philippines, LM19-Native Variety, showed resistance to the disease. It can be utilized for further mungbean breeding programme in Thailand [9].

Novel mungbean germplasm derived from induced mutation

Variegated leaf

Variegated mutant was obtained from Gamma-rays irradiation at a rate of 500 gray. All F_1 plants from the cross between the variegated mutant and the normal leaf parent showed normal green leaves without reciprocal, while the F_2 plants segregated well in a 3 : 1 ratio. The number of F_3 lines showing all green plants, segregating, all variegated plants fitted well with the 1 : 2 : 1 ratio. Thus it can be inferred that the variegated leaf character is controlled by a single recessive nuclear-encoded gene. We propose *var1* as the gene symbol [10].

Multiple leaflet

Two new multiple leaflet mungbean mutants were also obtained from Gamma-rays irradiation. A mutant with small pentafoolate was crossed with a large heptafoolate mutant to study the inheritance at Kasetsart University, Kamphaeng Saen Campus. AFLP markers were also screened

to make a partial linkage map around the genes controlling multifoliolate leaflets. The number of F_2 plants was tested against a 3, 1 ratio for segregation in a single locus and 9 : 3 : 3 : 1 for 2 independent loci using the Chi-square goodness of fit test. The heterogeneity among the F_2 families was also tested accordingly. Crossing between 7 large leaflet (L-7) and 5 small leaflet (S-5) mungbean mutants resulted in the normal-trifoliolate (N) F_1 . The F_2 can be classified into number of leaflet per leaf and leaflet size with large-trifoliolate (N_1-N_2 -), small-pentafoolate ($N_1-n_2n_2$), large-heptafoolate ($n_1n_1N_2$ -), and small-heptafoolate ($n_1n_1n_2n_2$) at the dihybrid ratio of 9 : 3 : 3 : 1. The finding is thus evident that leaflet number was controlled by n_1 locus and leaflet size was controlled by n_2 locus of genes, respectively. However, all three AFLP markers associated with leaflet number in this study corresponded to n_1 locus only. The n_2 locus can have a pleiotropic effect upon the leaflet size such that the N_2 allele controls large leaflet size as well. Another hypothesis is that the n_2 locus might be closely linked with the *s* locus so that there was no progenies with large pentafoolate leaflet (hypothetically carrying $N_1-n_2n_2S$ -genotype). [10]

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Development of Mutant Varieties of Crop Plants at NIAB and the Impact on Agricultural Production in Pakistan

M A Haq

Abstract

The Nuclear Institute for Agriculture and Biology is the prime institute of Pakistan Atomic Energy Commission in the agricultural sector. It began to function in 1969. The main objective of the institute is to conduct research in agricultural and biological problems, especially in those areas where nuclear techniques have an edge over conventional methods. The institute has been conducting research and development work related to crop improvement through mutation breeding. Mutation breeding involves the use of induced beneficial changes for practical plant breeding purposes both directly as well as indirectly. The main objectives have been to confer specific changes such as improvement of plant architecture, earliness in maturity, resistance against diseases and pests, and improved physiological characters, i.e. heat tolerance, cold tolerance, uniform maturity, photoperiod insensitivity etc., in the native well adapted crop varieties/exotic lines to make them more productive. The use of induced mutations for crop improvement has led to the development of 24 improved varieties of different crops at NIAB which clearly indicates the potential of this technique. In addition, a wealth of genetic variability has been developed for use in the cross breeding programmes and a few varieties of cotton and chickpea have been developed in Pakistan by using induced mutants as one of the parents. These improved crop varieties in Pakistan have played a significant role in increasing agricultural production with positive impact on the economy of the country. The estimated additional income accounted by the selected varieties of NIAB was 1.744 billion US dollars up to 2005.

Introduction

Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad is a prime agricultural institute of Pakistan Atomic Energy Commission. NIAB is one of the four agricultural research centers of the Commission and it now constitutes an important element in the infra structure of scientific research in the country. NIAB has made an indelible mark in the field of agricultural research in the country. Its output in terms of both basic and applied research has more than justified the modest expenditure on its establishment and operation. The main objective of NIAB is to promote peaceful applications of atomic energy in the biological research in general and agricultural research in particular. It was established to demonstrate how modern and advanced nuclear techniques could contribute to major improvement in agricultural output both in quantity and quality, and thereby bringing the economic benefits of atomic energy to the people of Pakistan, 80% of whom earn their livelihood through agriculture.

By a very careful selection of research projects, NIAB has concentrated on devising methods and evolving crop varieties for increasing agricultural productivity and on conservation of inputs and produce. It has been able to make a significant contribution not only in agricultural

research but also in understanding some relevant basic biological processes. It has clearly established a role for radiation induced mutations in crop improvement and has succeeded in evolving better germplasm of selected key crops. Work on improvement of cotton, rice, and grain legumes is in progress through the use of mutation breeding, conventional breeding and wide hybridization techniques. The prime strategy in mutation-based plant breeding has been to upgrade the well-adapted varieties by altering one or two major traits. These include characters such as plant height, maturity, seed shattering, and disease resistance, which contribute to increased yield and quality traits [1]. In several mutation-derived varieties, the changed traits have resulted in synergistic effect on increasing the yield and quality of the crop, improving agronomic inputs, crop rotation and consumer acceptance [1]. The breeding efforts have resulted in the development of 24 improved varieties of crop, including six varieties of cotton, two of rice, four of chickpea, 10 of mungbean and two of lentil. In addition, many mutants of these crops are at advanced stages of testing.

In the present paper the development of induced mutants and mutation-derived varieties at NIAB is discussed and their economic impact presented.

Materials and Methods

The research work at NIAB was started during 1969. In crop improvement programme the self-pollinated crops i.e., cotton, rice, chickpea, mungbean and lentil were selected. Mutation breeding involves the use of induced beneficial changes for practical plant breeding purposes, both directly as well as indirectly coupled with the conventional hybridization in some cases. The main objectives have been to confer specific changes such as improvement of plant architecture, earliness in maturity, resistance against diseases and pests, and improved physiological characters i.e. heat tolerance, cold tolerance, uniform maturity, photoperiod insensitivity etc., in the native well adapted crop varieties/exotic lines to make them more productive. The strategy in mutation breeding was to upgrade the well-adapted varieties by altering one or two major traits. Gamma radiation treatments and different EMS concentrations have been tried depending upon radio sensitivity tests. As a result of these breeding efforts different varieties of these five mandate crops have been developed at NIAB.

Results and Discussion

The efforts at NIAB have resulted in evolving 24 improved varieties of cotton, rice, chickpea, mungbean and lentil (**Table 1**). These self-pollinated crops were selected because of little variability available in them to improvement through conventional techniques. The area and additional income from selected varieties of NIAB during 2004-05 is given in **Table 2**. Some of the salient results have been described here.

Cotton

The mutation breeding programme in cotton was initiated in 1970 to create genetic variability for development of early maturing, high yield-

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ing varieties and germplasm having resistance to diseases, insects and other stresses with better fiber qualities.

Crosses were made between a local (AC 134) and an exotic variety (Delta pine) followed by the irradiation of F_1 hybrid. From the selections, line NIAB 78 had the desired fiber quality and gave the highest yield. It was released as a commercial variety in 1983. It has proved to be adapted to the different agroclimatic areas of the country. Since its introduction cotton, production registered a quantum jump in Pakistan [2]. The cumulative additional income to the farmers because of this variety was 612.4 million US dollars from 1882-83 to 2005 (Table 2). The insect resistant and high yielding variety NIAB 86 was evolved as the result of a cross between a pest resistant mutant H 1-9-6-2 and a long staple mutant SP 16. It was released as commercial variety in 1990. Crossing of mutant line with an exotic germplasm having nectariless leaf resulted in the development of NIAB 26 which was released in 1992.

The onset of CLCuV disease in early nineties resulted in a sharp decline in cotton production inflicting severe set back to our economy. This necessitated incorporation and/or induction of CLCuV resistance into the adapted cotton cultivars. Besides induction of mutations, various sources of CLCuV disease resistance tried to achieve the desired objectives. A nectariless line that had tolerance to heat and leaf curl virus disease was released as a commercial variety 'NIAB Karishma' in 1996. NIAB Karishma brought farmers US \$ 294.4 million additional income since 1997-98 to 2005. Moreover, a number of elite mutants viz., NIAB-92 (Stoneville), NIAB 313/12 (*G. barbadense* x *G. hirsutum*), Hi (PIMA x *G. hirsutum*), St-3, Hi-9, M 555, Mutant Chandni and Mutant 39 have been developed with specific characteristics of economic value. These mutants proved an asset as a number of varieties viz. Shaheen, NIAB-86, CIM-109, SLS-1 and CRIS-9 have been evolved by various organizations in the country by employing these mutants in their breeding programme [3].

Mutant NIAB-999 has been derived from H1 x LRA 5166 cross combination followed by 300Gy of F_1 seed. It has heat resistance, early maturity, high yield and wider adaptability. Its earliness makes it suitable to fit in wheat-cotton-wheat rotation system. It was approved for general cultivation during 2003. Another mutant strain NIAB-111 has been derived from the intraspecific hybridization, NIAB-313/12 (ISM) x CIM-1100 followed by F_1 seed treatment with 300Gy. It is a high yielding, CLCuV resistant, superior fiber and heat tolerant variety of cotton approved for general cultivation during 2004.

Rice

Rice is the third largest crop after wheat and cotton in Pakistan. It is high value cash crop and is also major export item. It accounts for 5.7% in value added in agriculture and 1.2% in GDP. It is grown on an area of 2,621 thousand ha producing 5,547 thousand tons with paddy yield of 2116 kg ha⁻¹. Pakistan grows enough high quality rice to meet both domestic and export demands. Different varieties of rice are grown in Pakistan, viz. Super Basmati, Basmati 385, Irri-6, Niab-Irri-9, KS-282 and KS 133, etc. Pakistan is primarily known for its aromatic rice. Two types of rice dominate the market: Basmati, which is mainly grown in the Punjab province and Irri types, which is mainly grown in Punjab and Sindh provinces. Genetic diversity for early maturity, disease tolerance, semi-dwarfism etc. needs to evolve in Basmati rice for its sustainable production. The IRRi varieties introduced in Pakistan have higher yields than the traditional cultivars but their grain quality does not meet the consumers' demand.

A research programme was initiated at NIAB for creation of genetic variability in Basmati and IRRi background through induced mutations and hybridization. The objectives for Basmati improvement were to evolve early maturing, short statured, high-yielding, good quality and salt tolerant lines while grain quality improvements were desired in the IRRi varieties.

Earlier research efforts resulted in the release of an early maturing mutant Kashmir Basmati (derivative of Basmati 370) as a variety for general cultivation in Azad Kashmir during 1977. A number of short statured mutants were also developed [4,5]. Several semi dwarf mutants were selected from basmati varieties and used in genetic studies [6]. Studies to identify new gene sources of early maturity and dwarfism in the adapted Basmati background revealed that early maturity gene in Kashmir Basmati was non allelic to that in variety C-622. Similarly, the gene for dwarfness in mutant DM 107-4 was found non allelic to that in IR-6 [7]. The information thus obtained was utilized in the development of short statured and early maturing varieties of Basmati rice. Among other mutants, NIAB-6 proved to be salt-tolerant producing high yields at salinity levels of ECe 10 dSm⁻¹. NIAB-6 was approved and designated as "Niab-Irri-9" by the government of Punjab during 1999 for general cultivation. Niab-Irri-9 is a non-aromatic, fine grain, salt tolerant and high yielding mutant line (derived from IR-6). This variety occupies about 64% area under non-aromatic rice in Punjab and gave 2.43 million US dollars additional income to the farmers during 2004-05 and 16.18 million US dollars from 1999-2000 to 2005 (Table 2). Some of extra long grain mutants namely EL-30-2-1, EL-30-2-2 in Basmati Pak background having grain length and width of 10 and 1.60 mm respectively and elongation of more than 18 mm are being utilized in cross breeding and a number of recombinants with desired traits have been selected.

Chickpea

Pakistan ranks second in terms of acreage and third in terms of chickpea production. With protein content nearly twice that of cereals, it is a cheap source of quality protein that complements the proteins in cereals thus enhancing the nutritional value of cereal-dominated diet. Chickpea is of two main types, desi and kabuli. Both are botanically similar, but there are strong consumer preferences in one or the other. The contribution of kabuli to the production is 15%, which is declining due to its greater susceptibility to various stresses than desi type. As a result the price of kabuli remains high and we have to spend more than 250 million rupees annually on its import. In Pakistan, the crop is grown on more than 1.0 million hectares with an annual production of 0.58 million tones. Low yield is due to low yield potential of land races and poor crop management. *Ascochyta* blight and *Fusarium* wilt diseases are also major constraints to its production. To develop high-yielding, widely adapted and disease resistant varieties, a programme at NIAB was initiated in 1974, where an integrated approach to chickpea improvement is being pursued. A high priority has been given to screening of segregating material and advanced mutants for *Ascochyta* blight and *Fusarium* wilt resistance. Yield testing of new mutants/varieties is conducted in cooperation with various national and international agencies.

Every year at NIAB, seeds of at least two chickpea genotypes (one desi and one kabuli) are treated with mutagens for the creation of genetic variability. During last 34 years, 29 genotypes/ varieties have been treated with at least two doses of gamma irradiation and ethyl methane sulphate (EMS). Mutation breeding requires an effective mutagen treatment and an efficient way of selection [8]. For gamma radiation treatments, dose range of 150 to 750Gy and for EMS treatments, concentrations ranging from 0.1-0.5% have been tried depending upon radio sensitivity tests.

An achievement of these efforts has been the evolution and release of an *Ascochyta* blight resistant and high-yielding variety of chickpea namely cm 72 in 1983. Air-dried seed of blight susceptible but high yielding genotype 6153 were exposed to gamma radiation treatment of 150Gy in 1974; selections made in M_2 - M_3 generations [9]. This mutant cultivar covered more than 40% chickpea growing area in the country in 1987 and has helped greatly to stabilize chickpea production.

As a result of efforts to induce blight resistance in different genetic backgrounds other than 6153 to produce alternate sources of resistance,

a blight resistant and wilt tolerant mutant cm 88 was derived from variety C 727 and released in 1994. Air-dried seed of C 727 were exposed to gamma radiation treatment of 100Gy in 1977-78. The M₂ was screened in the *Ascochyta* Blight Nursery (ABN) in 1978-79 and resistant plants were selected. The resistant plant progenies were further evaluated in the ABN and also evaluated for various morpho-agronomic traits and yielding ability in Preliminary, Cooperative and National Yield Trials in the successive generations. cm 88 proved to be higher yielding and resistant to *Ascochyta* blight and *Fusarium* wilt [10].

K850 is a bold seeded high yielding variety but it is highly susceptible to *Ascochyta* blight. Efforts were therefore made to rectify this through induced mutation. Seeds of this variety were treated with different doses of Gamma-rays and screening for disease resistance in M₂ generation was done in the nursery by artificially creating disease epiphytotic conditions. Selected progenies of resistant M₂ plants were studied for agronomic characteristics and samples of each line were tested for disease resistance. Based on yield potential, bold seed and *Ascochyta* blight and *Fusarium* wilt resistance; cm 31-1/85 was selected and released as a variety cm-98 in 1998 [11]. The release of this variety meets the consumer's demand/ preference for large seed size variety.

Large scale cultivation of these high yielding and disease resistant varieties has helped greatly to stabilize/increase chickpea production in the country and since their release no serious blight epidemic has been reported [11].

Many good sources of *Ascochyta* blight resistance are available in the exotic germplasm especially from ICARDA. However, they are not well adapted in Pakistan and are badly affected by *Fusarium* wilt. The breeding work on kabuli chickpea has resulted in the release of a high-yielding and disease-resistant variety cm2000 in 2000. This variety has been evolved by creating genetic variability in an exotic variety ILC 195 using 150Gy gamma radiation [12]. It is recommended for cultivation in rainfed and irrigated areas of Punjab. Large-scale cultivation of cm 2000 greatly helped in increasing the kabuli chickpea production in the country.

The current area covered by these mutant cultivars is more than 30% of the total area under chickpea. The additional income to the farmers has been estimated at 11.76 million US dollars during 2004-05 and 752.3 million US dollars from 1997-98 to 2005 (Table. 2).

To induce resistance against wilt disease in susceptible kabuli variety (Pb.1), seeds were treated with different doses/concentrations of physical and chemical mutagens and screening for wilt resistance in M₂ generation was done in the natural wilt sick field. From M₂ segregating populations and subsequent generations cm94/99 was selected and evaluated in various yield trials. This mutant exhibited 38.0 and 66.8% increased yield than check variety cm2000 in Chickpea National Uniform Yield Trials (CNUYT)- kabuli during the years 2003-04 and 2004-05, respectively. The candidate line cm94/99 was cleared by the technical experts in the spot examination during March, 2008 and will be discussed in the meeting of Punjab seed council for approval as a commercial variety for general cultivation.

As a result of mutation breeding efforts, an excellent asset of genetic variability has been created in both types (desi and kabuli) of chickpea through induced mutations. More than 600 disease-resistant (*Ascochyta* blight, *Fusarium* wilt) and morphological mutants affecting most parts, such as leaf, flower, plant height, plant type, pods, maturity and seed were selected from M₂ and subsequent generations. Most of the selected mutants have been confirmed for mutational traits and evaluated for various morpho-agronomic traits. The true breeding mutants have been added to the gene pool being maintained at NIAB, Faisalabad and PGRI, Islamabad. This variability is supplied as germplasm to various national and international organizations for use in breeding programmes. Interspecific hybridization between different cultivated varieties and annual wild *Cicer* species has provided useful genetic variability.

Mungbean

The work on mungbean improvement was initiated at NIAB in 1970s. The main objective of the mungbean improvement programme was to create genetic variability through induced mutations and hybridization, to evolve high-yielding and disease-resistant varieties having compact plant type, earliness and uniform maturity. Since then 10 high-yielding, early-maturing and disease-resistant varieties have been released. Of these, five varieties viz. NM 28, NM 13-1, NM 19-19, NM 20-21, and NM 121-25 are derivatives of small seeded, local adapted germplasm whereas the large seeded varieties viz. NM 51, NM 54 resulted from hybridization between exotic and local germplasm [3]. The most popular bold seeded variety NM 92 was released in 1996 and a medium seeded variety NM 98, having shiny seed coat color was released in 1998 [13]. During 2006, another bold seeded variety NIAB Mung 2006, a derivative of an exotic AVRDC accession VC 1560D and an adapted variety NM-92 having high seed yield, purple hypocotyls and stem, synchronous pod maturity, higher number of pods per plant, and resistance to *Cercospora* Leaf Spot (CLS) and Mungbean Yellow Mosaic Virus (MYMV) diseases has been approved by the Provincial Seed Council for growing in the irrigated tract of the Punjab province. Purple hypocotyle and stem colour can be used as morphological markers for varietal identification. Varieties NM 92, NM 98 and NM 2006 have been under cultivation on more than 70% of mungbean acreage in Punjab province. NM 92 and NM 98 was grown on an area of 181 thousand hectares and brought 7.56 million US dollars addition income to farmers in 2004-05 and 68.8 million US dollars from 1996-97 to 2005 (Table 2).

Lentil

The improvement work on lentil through induced mutations and conventional breeding techniques was started in 1986. Research efforts have culminated in the development of two lentil varieties. NIAB Masoor 2002, a high-yielding, disease-resistant and early-maturing variety was released for cotton based cropping pattern. NIAB Masoor 2002 matures one month earlier than Masoor 93, and farmers of cotton area can grow cotton easily after the harvest of this variety. It is the result of hybridization between an exotic Argentinian variety Precoz and a local cultivar Masoor 85. Punjab Seed Council has approved this variety in the year 2002 for cotton growing areas of the Punjab province.

Another high-yielding and disease-resistant variety, NIAB Masoor 2006, has been developed through induced mutation (ILL 2580 exposed to 200Gy) and was approved by Punjab Seed Council for traditional lentil growing areas of the Punjab province during 2006.

The discovery that ionizing radiations and chemical mutagens can cause genetic changes and modify linkages offered promise to the improvement of crop plants. Mutation breeding involves the use of induced beneficial changes for practical plant breeding purpose both directly as well as indirectly. Mutation breeding can be used to complement and supplement existing germplasm resources [14]. The prime strategy in mutation-based plant breeding has been to upgrade the well-adapted varieties by altering one or two major traits and these include characters such as plant height, maturity, seed shattering, and disease resistance, which contribute to increased yield and quality traits [1]. Induced mutations have been used in the improvement of major crops such as wheat, rice, barley, cotton, peanuts, beans, which are seed propagated [15]. More than 1,800 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in 50 countries [16]. Among the mutant varieties released, cereals are at the top followed by legumes, demonstrating the economics of the mutation breeding techniques.

In Pakistan, the use of mutation breeding technique for the improvement of crops has led to the development of 59 cultivars of cotton, rice, wheat, chickpea, mungbean and rapeseed which have played a significant role in increasing crop production in the country.

Conclusion

The use of induced mutations for crop improvement has led to the development of 24 improved varieties of different crops at NIAB which clearly indicates the potential of this technique. In addition, a wealth of genetic variability has been developed for use in the cross breeding programmes and a few varieties of cotton and chickpea have been developed in Pakistan by using induced mutants as one of the parents. These improved crops varieties in Pakistan have played significant role in increasing agricultural production with positive impact on the economy of the country.

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Socio-Economic Impacts of Mutant Rice Varieties in Southern Vietnam

K T Do

Abstract

Rice production plays an important role in the socio-economic development of Vietnam, especially in the **Mekong River Delta** (MRD) region, which is responsible for more than half of the total and 90% of the national rice export. Before 1995, no **mutant rice varieties** (MRVs) were cultivated in the MRD. At present, rice variety improvement is the main focus of the national breeding programme and 8 rice mutants have been developed, occupying 10.3% of the total modern varieties in Southern Vietnam. The mutant varieties developed so far have better resistance to lodging, disease and insect damages, higher tolerance to soil stresses such as acid sulphate soil, drought etc., and also exhibit earliness and higher yield potential. Some of the best mutant varieties, namely VND95-19, VND95-20, VND99-3, TNDB-100 have already been released for large-scale production in the MRD. VND95-20 has become one of the top 5 exported varieties and is grown annually on more than 300,000 ha in Southern Vietnam.

Some of these mutants have given promising recombinants through hybridization and in particular the varieties VN121, VN124, OM2717 and OM2718 have been released into production. A successful combination of aromatic characteristics, short duration, high yield, tolerance to new diseases (GSV & RSV) and insects (BPH), and consequent reduction of spraying times of pesticide per crop, have greatly benefitted health & environmental protection.

During the past 8 years under the IAEA Technical Co-operation (TC) project, the total cultivated area of MRVs in Southern Vietnam has been more than 2.54 millions ha. Until 2008, the 8 rice mutant varieties produced an added return of 374 million USD over the previous years and continue to produce added return for farmers. More specifically, VND95-20, VND99-3, TNDB100, VND95-19, OM2717, OM2718, VN121 and VN124 returned 300.00, 9.0, 37.5, 6.0, 12.0, 8.4, 0.8 and 0.7 million USD, respectively. The application of MRVs reduced spraying times per crop two- to three-fold due to their tolerance to diseases & insects.

MRVs are used in the strategy programme '*Eradicate hunger and alleviate poverty*' of different national projects, particularly for the ethnic minorities in mountainous and remote regions of Southern Vietnam. Due to the significant contribution of MRVs to socio-economic development, their development has received many prizes by the national & local Government.

Introduction

Vietnam is an agricultural country and 73.5% of its population lives in rural areas. In 2006, Vietnam's Gross Domestic Product (GDP) was valued at 973,790 billion VND (Vietnamese dong) (at current prices, equivalent to 60.86 billion USD). Agriculture & forestry GDP shared 12.39 billion USD, which occupied 20.36% of the total national GDP [1].

Rice plays an important role in the socio-economic development of Vietnam. The country has made great achievements in paddy production and has become the second largest rice exporting country in the world since 1989. Paddy production is a traditional activity of Vietnamese farmers, hence it always holds the central role in Vietnam's agriculture and socio-economic issues. The rice sector accounted approximately for 37.0% of agricultural GDP and 26.0% of the agricultural product export value between 2000-2004.

In 2006, the paddy land and sown areas were approximately 4.2 million ha and 7.3 million ha, respectively, occupying 16.9% of the agricultural and 65.3% of the annual crop land areas.

In 2007, total rice production was 36.0 million tons, of which 4.5 million tons was exported (valued at about 1.5 billion USD). The Mekong River Delta (MRD) produced 18.73 million tons (52% occupation) and exported 4.0 million tons (90% of the total rice export). Over the past 20 years, Vietnam has exported 60 million tons of rice to different continents of the world, averaging 4.5 million tons per year for the period between 2002-2007. This has been achieved thanks to the reformed Government policy and the innovative technologies used. Breeding of modern varieties has played an important role in agriculture and increasing the income for farmers.

Between 1987-2006, 78 rice varieties were released in Southern Vietnam: 32 by introduction (41.0%); 33 by hybridization (42.3%); 8 by mutation (10.3%); and 5 by pure line selection (6.4%). Before 1995, there were no mutant rice varieties in production, in Southern Vietnam. Consequently, the rice varieties delivered solely by introduction and hybridization could not meet the production requirements, particularly concerning varieties having traits with high tolerance to adverse conditions, high yield & good quality.

Since 1992 rice mutation breeding combined with other methods has been undertaken, leading to the successful creation of mutant varieties with distinct characteristics, which have significantly affected the socio-economic issues of Southern Vietnam. Some of the best mutant varieties, VND95-19, VND95-20, VND99-3, TNDB-100, OM2717 and OM2718 have been released for large-scale production in the MRD region. Among them, VND95-20 has become one of the top 5 exported rice varieties and is grown on more than 300,000 ha in Southern Vietnam [2]. Some mutants gave promising recombinants in aroma, tolerance to BPH, Grassy Stunt Virus (GSV) & Ragged Stunt Virus (RSV) diseases. Selected varieties such as VN121, VN124 have been released into production in recent time [3].

Breeding, development and production of mutant varieties

Mutation breeding

Since 1993, under the IAEA Technical Co-operation (TC) project since 1997, the mutation breeding programme was initiated for rice. The breeding programme used introduced varieties (IR64, IR50404), local varieties (Nang Huong, Tam Xoan, Tai Nguyen and Tep Hanh) and mutants (VND31, VND22-36) as genetic material to be induced with

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mutations and combined with hybridization. After 5-6 years, the best mutants were released into production. Methodically, dry and germinated seeds were exposed to gamma rays of a ^{60}Co source at the Nuclear Research Institute, Dalat city, Southern Vietnam. The doses of 200 and 300Gy were applied for seed treatments. Populations of 10,000 – 15,000 M_1 plants were established and evaluated from M_2 to M_7 generations. The best lines were tested, released and approved as temporary and national varieties by the Ministry of Agriculture & Rural Development (MARD).

Development and production of mutant varieties

IR64 is good quality variety but has a rather long duration, and is not suitable for the wet season. VND95-20 is a mutant variety derived from IR64. The variety inherited the main good characteristics of IR64 and added some desired characteristics through mutation.

VND95-20 was released into production & approved as a national variety by MARD in 1999. Since 2000, VND95-20 has been developed in 21 provinces over 280,000 – 350,000 ha per year. The variety is cultivated in Dong Thap, Long An, Tien Giang, Can Tho, Tra Vinh, An Giang, Vinh Long (Mekong River Delta), Dong Nai, Lam Dong, Tay Ninh, Baria-Vung Tau (East-Southern VN), Daklak and Gialai (Highland area). Recently, VND95-20 has been the key variety for production and export. Advantages of this mutant variety is short duration 90-100 days (7 days shorter than the original variety IR64), high adaptation and can be cultivated in diverse seasons and locations. Generally, mutant varieties are tolerant to intermediate acidic soil. This characteristic is very important because the acidic soil in the Mekong River Delta region covers more than 41% of the total cultivated land.

VND95-19 (another mutant from IR64) has high yielding potential (can yield 11 tons /ha), high tolerance to acidic soil and adverse conditions. The variety is resistant to Brown Plant Hopper (BPH) and Blast disease (BL), and is developed in several provinces: Soc Trang, Bac Lieu, Kien Giang, Dong Nai and the Highland area [4]. During 1997-2000 its cultivated area covered about 20,000ha per year. However, due to high chalkiness, the variety is only for domestic consumption.

VND99-3 is a mutant variety obtained from the Nang Huong variety, a local aromatic variety. The Nang Huong variety has long duration, low yield, photoperiodic sensitivity and is limited in production. VND99-3 was approved as a national variety in 2006. It is highly accepted due to having short duration (92-100 days), high tolerance to adverse conditions such as acid sulphate and drought conditions in Southern Vietnam. These improvements combined with iron toxicity tolerance, show that the mutant variety has inherited desirable traits from the parent local variety and shows similar characteristics or better than the original variety. Presently VND99-3 covers 15,000-18,000 ha per year of cultivated area in Southern Vietnam and continues to spread widely in Southern Vietnam [5].

TNDB100 is an induced mutation from the Tai Nguyen local variety, generated by gamma rays. The variety has very short duration (95-100 days), good quality, high yield (5-8t/ha) and intermediate tolerance to BPH and BL. TNDB100 was released by Cuu Long Delta Rice Research Institute (CLRRI) in 1997 and developed 50,000 ha/year during 2000 – 2005 [6].

VN121 is a new variety which was generated from mutation induction combined with hybridization. VN121 is widely accepted by farmers, because of its characteristic short duration, high yield, good quality (aroma, long grain, no chalkiness) and its tolerant to BPH, BL and GSV. The variety has been expanded in some Southern provinces: Tien Giang, Long An, Ba Ria - Vung Tau, Dong Nai, Tay Ninh & Dak Lak.

The VN124 variety has also been accepted for production due to its very short duration, aroma, good quality for export, tolerant to BPH, BL and GSV. The variety has been sharply expanded in Long An, Tay Ninh, Dong Nai and other provinces.

Socio-economic and environment impacts

Socio - economic impacts

Before 1995, in Southern Vietnam, many had thought that mutation breeding had very little hope and also that mutant varieties could not be stable in production. Through the developments of recent years, however, it has been proven that mutation breeding is a very good way to obtain novel varieties and that mutant varieties have a prolonged production life (more than ten years in the case of VND95-20). Nowadays, through practical approaches, many leaders and junior scientists have changed their doubtful thinking and have started to believe in the significant role of mutation breeding.

In general, mutation breeding has the advantage of saving 30% of the breeding programme time compared to hybridization techniques, especially in local varieties. While breeding new varieties takes 8-9 years or more, new varieties through mutation breeding takes only 5-6 years [7]. Mutation breeding is a very useful tool that can be applied in institutions that lack infrastructure such as green houses, field areas etc.

The rice mutation breeding programme of IAS and CLRRI have an annual budget of about 20,000 USD, so 300,000 USD from 1992 up to 2007. A quick calculation for the variety VND95-20 that is cultivated on average over an area of 250,000 ha (from 200,000-340,000 ha/year) x 8 years (from 1999 - 2007) x a yield of 6.0t/ha x 10% added return (in practical production it gets a higher yield than 10%) x 4 million VND/ton of rough rice (VND/t is price for ordinary paddy) gives us 4,800 billion VND (equivalent to 300 million USD). Exported rice is usually 20-25% higher in price than the ordinary domestic rice.

A similar calculation for VND99-3's added return, 15,000 ha x 4 years x a yield of 6.0t/ha x 10% added return x 4 millions /t gives us 144 billion VND (equivalent to 9.0 million USD). In the case of the varieties like TNDB100, VND95-19, OM2717, OM2718, VN121 and VN124 the produced added return reaches 37.5, 6.0, 12.0, 8.4, 0.8 and 0.7 million USD, respectively (Table 1). Support from international programmes (IAEA and others) and investments in the rice project of the IAS and the CLRRI was about 400,000 USD. With an added return of 374.0 million USD from 8 mutant varieties in Southern Vietnam makes this project a very effective investment. For poor countries, the influx of money depends greatly on agricultural activities, at a significant level, particularly for farmers and rice exporters. These mutant varieties will continue to produce valuable return in coming years. For the past 8 years, the total sown area of the mutant varieties has been about 2.45 millions ha in Southern Vietnam.

Due to their high adaptation in large-scale production, different seasons and tolerance to adverse conditions, the mutant varieties were the main varieties selected in many national projects of "Eradicate hunger and alleviate poverty" programmes of the Vietnam Government in Southern Vietnam. These mutant varieties have contributed food security for the ethnic minorities such as Ragley, St'ieng, Nung, K'ho, Kh'me leading to better condition for forest protection in mountainous regions.

For example, the large number of Ragley ethnic people in the Khanh Dong hamlet, the Khanh Vinh district and the Khanh Hoa province usually have the conventional habit to collect forest products and exchange them for food as they do not know cultivation. Every year, the local government offers assistance for food and necessary items to the inhabitants, but it can not resolve the basic problem because hunger and poverty remain. To resolve the problem, we cooperated with local collaborators to conduct a project for technology transfer including the adoption of new rice varieties by the poor ethnic groups during 2003-2005. After 2 years into the project, ethnic people had cultivated VND95-20 and VND95-19 with high yield (6.4 t/ha) in comparison to other varieties that yielded only 4.4t/ha. Due to VND95-20's high adaptation, and good quality, local people preferred to grow this variety for two crops per year. Consequently, local people escaped from hunger, poverty diminished

and deforestation stopped. Now they have a sustainable base to improve living conditions, beginning from having enough and surplus food.

Another example related to mutant rice varieties was the 2006-2007 outbreak of the Grassy Stunt Virus disease in the Southern areas, transmitted by Brown Plant Hopper. The Vietnam Government organized a campaign to mobilize different actions to control the pest. Our role was to transfer new tolerant varieties into production. In some provinces we supplied new varieties (including mutant as VND95-20, VND99-9, VN121 and VN124) and guided the production procedure in adverse conditions. As a result, in the Tay Ninh province and the Ben Cau district during the rainy season of 2007, the mutants yielded on average 5.1 t/ha compared to the local varieties that yielded only 4.1t/ha. Due to farmers having reduced insecticides, fertilizers and seed rate with a total spending reduction of 27,0% (about 175 USD/ha) in the production model of the mutant varieties, the added return from the use of mutants increased by 437.5 USD/ha over the concurrent ordinary varieties.

Environmental impacts

Mutant varieties have high tolerance to insects and diseases, so pesticides were applied two- to three-fold less during production in comparison to that for susceptible varieties. This not only saves investment capital and improves the safety of the rice products, but also strongly protects human and animal health.

Economic impacts

- Over the past 8 years, the total cultivated area of MRVs in Southern Vietnam has reached about 2.54 million ha.
- The mutant varieties contributed for production have produced added returns of (in millions USD): VND95-20 (300.0); VND 99-3 (9.0); TNDB 100 (37.5); VND95-19 (6.0); OM 2717 (12.0); OM 2718 (8.4); VND 121 (0.8) & VN124 (0.7).
- 8 mutant varieties contributed an added return of 374 million USD over the past years and will continue to provide valuable return for farmers in future crops.
- The new mutant varieties are tolerant to new diseases and insects, have replaced susceptible varieties and will continue to have a significant impact on sustainable rice cropping systems in the future.

Innovative techniques of MRVs

- Higher yield of more than 10% in comparison to control varieties.
- Very short growth duration: MRVs can be cultivated for 2-3 crops per year, escaping early flooding.
- Tolerance to adverse conditions, large adaptation allowing MRVs to be cultivated in different areas (acid soil, alluvial, affected salinity, wet season).

Table 1. Mutant rice varieties & their economic impacts in Southern Vietnam

No.	Mutant variety	The year of release	Cultivated area since release (ha)	Added return since release time (million USD)	Main superior characters
1	VND95-20	1999	2,000,000	300	Large-scale adaptation, good quality,
2	VND 99-3	2004	60,000	9.0	Tolerant to acid soil, good plant type
3	TNDB100	1997	250,000	37.5	Good quality, short duration
4	VND95-19	1999	50,000	6.0	Tolerant to acid soil, good plant type
5	OM2717	2004	100,000	12.0	Tolerant to BPH, short duration
6	OM2718	2004	70,000	8.4	Tolerant to BPH, short duration
7	VN121	2007	5000	0.8	Aromatic, tolerant to BPH, GSV
8	VN124	2007	5000	0.7	Aromatic, tolerant to BPH, GSV
Total	-	-	2,540,000	374.4	-

Prizes for achievement from mutant varieties in Southern Vietnam
Due to the significant contributions to socio-economic development, the development of mutant rice varieties has received many prizes by from national & local governments and they are as follows :

1. First prize and Second prize in the Technology Creative Competition in Ho Chi Minh City, 1998
2. 2 Gold medals in the Fair of “ International Green Week” exhibition in Vietnam, 2000
3. 1 Golden Panicle Prize in the International Agriculture Fair organized in Can Tho, 1999
4. The National Prize on Science & Technology for Significant Contribution for Socio-economic issues of the country, 2005
5. Prizes for 30 Typical Science & Technology Achievements during the 30 years of the HCM City, 2005
6. The Golden Cup for Agriculture Products contributed to the country, National Agriculture Fair in 2006
7. The Creative Labor medal awarded by the Vietnam government, 2007

Conclusion

In brief focus, some significant impacts of the mutant rice varieties in Southern Vietnam are described below.

- Successful combination of aromatic characteristics with short duration, high yield, tolerant to diseases (BL, GSV & RSV) and insects (BPH).
- Shorter breeding times, reaching 30% reduction compared to hybridization methods.
- Confirmation that doses of gamma ray ⁶⁰Co treatment induces high mutation.

Socio–Environmental impacts

- MRVs are resistant to insects & diseases: Two- to three-fold reduction of spraying times per crop, saves production investment capital and protects from pesticide over-usage.
- Contributed to the strategy programme of “Eradicate hunger and alleviate poverty” and “Deforestation reduction” of different national and local projects, particularly for the ethnic minorities in mountainous and remote areas.

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