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*Review*

# Global impact of mutation-derived varieties

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**Summary**

During the past seventy years, worldwide more than 2250 varieties have been released that have been derived either as direct mutants or from their progenies. Induction of mutations with radiation has been the most frequently used method for directly developed mutant varieties. The prime strategy in mutation-based breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits, which limit their productivity or enhance their quality value. In this paper, the global impact of mutation-derived varieties on food production and quality enhancement is presented. In addition, the economic contribution of the selected mutant varieties of rice, barley, cotton, groundnut, pulses, sunflower, rapeseed and Japanese pear is discussed. 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. In contrast to the currently protected plant varieties or germplasm and increasing restrictions on their use, the induced mutants have been freely available for plant breeding. Many mutants have made transnational impact on increasing yield and quality of several seed-propagated crops. Induced mutations will continue to have an increasing role in creating crop varieties with traits such as modified oil, protein and starch quality, enhanced uptake of specific metals, deeper rooting system, and resistance to drought, diseases and salinity as a major component of the environmentally sustainable agriculture. Future research on induced mutations would also be important in the functional genomics of many food crops.

## Introduction

The discovery that X-rays induced mutations in the fruit fly *Drosophila melanogaster* (Muller, 1927) and in barley (Stadler, 1928) initiated a new field – induced mutagenesis, which later was to become the most important tool in locating genes on chromosomes, studying gene structure, expression and regulation, and for exploring genomes. Soon after this discovery many plant breeders and geneticists started to investigate the use of radiation-induced mutations for changing plant traits.

During the past seventy years, more than 2,252 mutant varieties have been officially released (Maluszynski et al., 2000). Of these, 60% were released from 1985 onwards. Most mutant varieties were released in China (26.8%), India (11.5%), USSR and Russia (9.3%), the Netherlands (7.8%), USA (5.7%) and Japan (5.3%). Many induced mutants were released directly as new varieties; others were used as parents to derive new varieties. For example, of the 2,252 varieties, 1,585 (70%) were released as direct mutants, i.e. from direct multiplication of a selected mutant and its subsequent release as a new variety. In rice, the majority of mutant varieties were developed as direct mutants selected from mutated populations. The remaining 667 crop varieties were derived through crosses with induced mutants. Mutation induction with radiation was the most frequently used method to develop direct mutant varieties (89%). The use of chemical mutagens was relatively infrequent. Gamma rays were employed to develop 64% of the radiation-induced mutant varieties, followed by X-rays (22%). Of the 2,252 accessions, 75% are in crops and 25% in ornamental and decorative plants. Most crop mutant varieties (1,603) were released in seed-propagated species, which include 1,072 cereals and 311 legumes (Maluszynski et al., 2000).

The prime strategy in mutation-based breeding has been to upgrade the well-adapted plant 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, e.g. modified oil profile and content, malting quality, and size and quality of starch granules. However, in many cases, the changed traits had a synergistic effect on the cultivation of the crop, agronomic inputs, crop rotation and utilization. For example, the short height genotypes in rice, wheat, barley and maize have contributed significantly to increasing grain yield because of their resistance to lodging and high planting density. The short height trait also allowed the use of relatively high doses of nitrogen application. The early maturity of some mutants resulted in timely planting of the follow-up crop; for example early maturity of cotton in Pakistan allowed early planting of the wheat crop, resulting in higher wheat yield. The induction of thermo-sensitive genic male-sterile mutant in *japonica* rice, which is controlled by a single recessive gene (Maruyama et al., 1991), contributed significantly to develop strategies for the production of hybrid rice varieties. Similar mutants have been induced by gamma rays in *indica* rice '26 Zhaizao' in China (Shen et al., 1993). Such mutants allow production of hybrid seed based on only two lines, and show increased yield from heterosis.

In this paper, the impact of induced mutants and mutation-derived varieties on increasing yield and enhancing quality is reported, and the economic contribution of selected mutant cultivars is presented. The role of induced mutants in developing functional genomics of major food crops is also discussed.

## Economic impact of a new mutant variety

The economic value of a new variety can be assessed from several parameters. These include:

- Area planted to the variety and percentage of the area under the crop in the region.
- Increased yield.
- Enhanced quality.
- Reduced use of pesticides and fungicides (e.g. in varieties resistant to diseases and insect pests).
- Savings in water (short duration of growth and drought tolerance).
- Increased land use through early maturity to facilitate crop rotation.
- Improved/intensified cropping systems with changed maturity or response to photoperiod.
- Improved processing quality and value of the products (e.g., oil, starch, malt, beer and whisky).
- Quality preference by the consumer (new flower and foliage colour in ornamentals, skin and flesh colour in root and tuber crops and fruit crops, aroma and glutinous nature in rice, and kernel colour in wheat).
- Increased nutritive value, high lysine and vitamins, increased oil-shelf life, reduced toxins.
- Increased yield of essential oils.
- New specialty and designer crops.
- Ease of harvest, threshing.
- Increase in export earnings.
- Reduction in imports.

Often, induced mutations lead to more advantages than a simple desired phenotypic change.

## Leading mutants of high value

Mutation-derived varieties have been released in 175 crop and plant species, including many important crops such as rice, wheat, cotton, rapeseed, sunflower, sesame, grapefruit and banana. Among these, some have made a major economic impact and include rice varieties in Australia, China, India, Pakistan and Thailand; cotton in Pakistan, Japanese pear in Japan, grapefruit in USA; barley varieties in Europe, durum wheat in Italy, sunflower in USA; sorghum in Mali and wheat varieties in North Western Frontier Province in Pakistan; groundnut and pulse crops in India, peppermint in USA, and ornamentals in India, the Netherlands and Germany. The economic contribution of selected mutant varieties is listed in Table 1. This value should not be taken as the absolute contribution of a mutated gene or of the mutant variety. The increased yield and enhanced quality of a new variety includes several other components such as its subsequent use for breeding, additional gains from heterosis in hybrid cultivars, response to increased agronomic inputs, and consumer preference. Hence, the direct economic value of a mutated gene in absolute terms cannot be determined. Genes function only in concert with all the other genes in a genome to alter the yield and quality of the end product.

*Table 1.* Economic impact of mutant varieties

| Crop | Country | Mutant variety | Basis of value assessment | Value or area |
|---|---|---|---|---|
| *Cereals* | | | | |
| Rice | Thailand | RD6 and RD15 | Total crop value at farm gate for the period 1989–98 | US$ 16.9 billion |
| | China | Zhefu 802 | Cumulative planted area between 1986–1994 | 10.6 million ha |
| | Japan | 18 varieties | Total crop value in 1997 | US$ 937 million |
| | India | PNR-102 and PNR-381 | Annual crop value | US$ 1,748 million |
| | Australia | Amaroo | Current annual planted area | 60–70% rice growing area in Australia |
| | Costa Rica | Camago 8 | Current annual planted area | 30% rice growing area in Costa Rica |
| | Vietnam | TNDB100 and THDB | Total planted area in 1999 | 220,000 ha |
| | Myanmar | Shwewartun | Total planted area in 1993 | 800,000 ha |
| Bread wheat | Pakistan | Jauhar 78, Soghat 90 and Kiran 95 | Additional income to farmers during 1991–99 | US$ 87.1 million |
| Durum wheat | Italy | Creso | Additional income to farmers during 1983–93 | US$ 1.8 billion |
| Barley | UK-Scotland | Golden Promise | Crop value (1977–2001) | US$ 417 million |
| | Numerous European countries | Diamant and derived varieties | Area planted in 1972 | 2.86 million ha |
| *Legumes* | | | | |
| Chickpea | Pakistan | CM 88; CM 98 | Additional annual income to the growers | US$ 9.6 million |
| Blackgram (urdbean) | India, Maharashtra State | TAU-1 | Value of increased production in season 1998–1999 | US$ 64.7 million |
| *Oil and industrial crops* | | | | |
| Cotton | Pakistan | NIAB-78 | Total value of crop from 1983–1993 | US$ 3 billion |
| | | NIAB-78 | Additional income to growers from 1983 onwards | US$ 486 million |
| Sunflower | USA | NuSun® | Grown area in 1994 | 50,000 ha |
| *Fruit trees* | | | | |
| Japanese pear | Japan | Gold Nijisseiki | Additional annual income to growers | US$ 30 million |
| Grapefruit | USA, Texas | Rio Star® | Grown area (year 2000) | 7,300 ha (75% of total area) |

## Value of mutant varieties

### *Cereals*

#### *Rice*

Rice is the major source of food for more than 50% of the global population and even more so in Asia. Mutation techniques have played a significant role in increasing rice production in the Asia-Pacific Region. According to the recent database (Maluszynski et al., 2000), 434 mutant varieties of rice have been released with improved characters such as semi-dwarf height, early maturity, improved grain yield, disease- and cold-tolerance, and improved grain quality. Of these, 225 (56%) were induced with gamma rays, 16 with X-rays, 7 with fast neutrons and 12 with other radiation sources. The majority of them (67%) were obtained as direct mutants (Maluszynski et al., 2001). Some rice mutant varieties have had considerable economic impact. Rutger has presented data on 11 mutant varieties, which were, or are grown annually on 100,000 hectares or more (Rutger, 1992).

The mutation $sd_1$ that determines short height in rice, similar to the spontaneous mutation $sd_1$ (Dee-geo-woo-gen in *indica* rice), was induced with gamma rays, and variety 'Calrose 76' was released in the USA (Rutger, 1992). This gene was transferred by crossing the mutant to other varieties, and resulted in the release of 20 new varieties in 3 countries. These included nine varieties – 'M7' (1978), 'M-101' (1979), 'M-301' (1980), 'S-201' (1980), 'M-302' (1981), 'Calmochi-201' (1981), 'Calmochi-202' (1986), 'M-103' (1990), and 'S-301' (1992) in California, nine varieties – 'Amaroo' (1987), 'Bogan' (1987), 'Echua' (1989), 'Harra' (1991), 'Illabong' (1993), 'Jarrah' (1993), 'Langi' (1994), 'Millin' (1995), and 'Namaga' (1997) in Australia, and two, 'Giza 176' (1989) and 'Sakha 101' (1997) in Egypt. Mutant variety Amaroo has been covering 60–70% of rice cultivation area in Australia, and on average yielded 8.9 t/ha grain with a potential of 13.3 t/ha (Clampett et al., 2001). As a result of the introduction of semi-dwarf varieties, the average yield of rice in Egypt increased to 8.9 t/ha compared with 3.8 t/ha of the world. Of these, 'Giza 176' became one of the leading varieties, with a potential yield of 10 t/ha (Badawi, 2001).

In Pakistan, the mutation induction in 'Basmati 370' resulted in the release in 1977 of a new variety 'Kashmir Basmati' which matures early and has cold tolerance, and yet retains the aroma and cooking quality of the parent. Conventional crosses derived from Basmati rice with non-aromatic varieties do not retain the aroma trait of the Basmati parent. Due to earliness and cold tolerance, Kashmir Basmati can grow at an altitude of 1000–1500 m, on areas not suitable for 'Basmati 370'. Hence, mutation induction has been a very specific method in the improvement of Basmati rice (Awan, 1991).

The Indian Agricultural Research Institute (IARI) has been a pioneer institution for research on induced mutations in the country since 1957, and has released many mutant varieties of crops and ornamentals. Several rice mutants induced with gamma radiation were released in India as high yielding varieties under the series 'PNR'; some of these were also early in maturity and had short height (Chakrabarti, 1995). Among, these, two early ripening and aromatic mutation-derived rice varieties, 'PNR-381' and 'PNR-102', are currently popular with farmers in Haryana and Utter Pradesh States. No data are available on the actual area planted to these varieties, since farmers and seed companies – once they purchase Foundation Seed – repeatedly multiply Certified Seed for several years. However, the following estimates could be made on the basis of data obtained from IARI. The rate of fresh seed replacement by farmers is only 10% per year. During the summer 2000, the Division of Genetics (IARI) produced and distributed 3,360 kg Breeders Seed of 'PNR-381', and 1295 kg of 'PNR-102'. Planted at a seed rate of 25 kg/ha and yield of 4 t/ha, this would produce 744,800 kg Foundation Seed and 119,168 tons of Certified Seed, sufficient for planting 4.76 million ha and producing 14.3 million tons paddy (at average yield of 3 t/ha) on farmers' fields. At the current support price of Rs.5500/t of paddy (the sale price of paddy is Rs.5000–6000/t), the value of rice (paddy) production from the two varieties at farm gate would be US$ 1,748 million per year. This does not include the value of the Certified Seed, which is sold at Rs. 5–6/kg, and has an additional value between US$ 13.2 million to 15.9 million. During 2000, India produced 132.4 million tons of paddy from 44.6 million hectares.

In China, twelve mutant varieties were officially released between 1962 and 1995 by the Institute of Nuclear Agricultural Sciences, Zhejiang Agricultural University. The Zhefu mutant varieties have been widely planted in the Zhejiang, Jiangxi, Hunan, Hubei, Anhui and Fujiang provinces, and the cumulative area under these varieties reached 14 million ha in 1995. The mutant variety 'Zhefu 802', induced by gamma rays in 'Simei No. 2', was the most extensively planted conventional rice variety between 1986 and 1994 in China; its cumulative planted area reached 10.6 million ha (Shu et al., 1997). The parent variety 'Simei No. 2' is also a mutant variety. The variety 'Zhefu 802' has the following characteristics: 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 some tolerance to cold.

In Japan, up to 1997, 18 mutant varieties of rice were released; some of them were resistant to lodging, and high yielding or with improved quality. According to a recent report of Science and Technology Agency, Japan, these varieties contributed US$ 937 million annually to the Japanese agriculture (Anonymous, 2000).

In Thailand, the work on induced mutations on rice was initiated in 1965 and stimulated in cooperation with IAEA. Two aromatic *indica* type varieties of rice, 'RD6' and 'RD15', were produced by gamma irradiation of a popular rice variety 'Khao Dawk Mali 105' ('KDML 105'), an aromatic and non-glutinous variety,

*Table 2.* Planted area, production and estimated value of rice mutant variety 'RD6' in Thailand between 1989–1998

| Season | Area planted (ha) | Production (t) | *Value in US$ |
|---|---|---|---|
| 89/90 | 2,474,129 | 4,234,391 | 529,298,875 |
| 90/91 | 2,478,437 | 4,283,072 | 535,384,000 |
| 91/92 | 2,227,476 | 4,023,965 | 502,995,625 |
| 92/93 | 2,433,512 | 4,073,735 | 509,216,875 |
| 93/94 | 2,356,684 | 3,801,540 | 475,192,500 |
| 94/95 | 2,330,135 | 4,195,266 | 524,408,250 |
| 95/96 | 2,429,362 | 4,343,549 | 542,943,625 |
| 96/97 | 2,557,924 | 4,528,222 | 566,027,750 |
| 97/98 | 2,524,576 | 4,599,995 | 574,999,375 |
| Total 1989–98 | 21,812,235 | 38,083,735 | 4,760,466,875 |

* Based on average sale price at US$125/ton (5 THB/kg) of paddy.

*Table 3.* Planted area, production and estimated value of rice mutant variety 'RD15' in Thailand during 1989–1998

| Season | Area planted (ha) | Production (t) | *Value in US$ |
|---|---|---|---|
| 89/90 | 169,324 | 305,730 | 38,216,250 |
| 90/91 | 182,912 | 324,244 | 40,530,500 |
| 91/92 | 327,723 | 580,652 | 72,585,250 |
| 92/93 | 144,182 | 230,888 | 28,861,000 |
| 93/94 | 227,123 | 388,885 | 48,610,625 |
| 94/95 | 272,258 | 519,309 | 64,913,625 |
| 95/96 | 278,488 | 530,079 | 66,259,875 |
| 96/97 | 284,947 | 466,711 | 58,338,875 |
| 97/98 | 253,007 | 538,575 | 67,321,875 |
| Total of 9 years | 2,139,964 | 3,885,073 | 485,637,875 |

* Based on average sale price at US$125/ton (5 THB/kg) of paddy.

grown in the North and North Eastern regions of Thailand. The parent variety KDML105 was released in 1926, and in spite of its low yielding ability, it is still one of the popular aromatic rice varieties because of its grain cooking quality. The mutant variety RD6 was released in 1977 and RD15 in 1978. Both varieties are the result of direct selection following treatment with gamma rays. Even 21 years after their release, these varieties are still grown extensively in Thailand. RD6 has glutinous endosperm and retained all the other grain characters including 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 rainy season in the wet areas.

According to the Bureau of Economic and Agricultural Statistics, Bangkok, during 1995–96, RD6 was grown on 2,429,361 ha, covered 26.4% of the area under rice in Thailand and produced 4,343,549 tons paddy (Anonymous, 1996; S. Chitrakon, pers. commun.). At the current sale price, THB 5,000/ton (1US$=40 THB), the farm gate value of paddy would amount to US$ 543 million for a single season, and after processing the value of rice would be US$ 1,740 million/year. Likewise, RD15 would add US$ 66 million to the paddy value at the farm gate; thus the two mutant varieties contributed US$ 609 million. After processing, 19.5 million tons paddy equals 12.5 million tons rice, which at the current price of US$ 625/ton in the local market, would be worth US$ 7,812.5 million per year. Thailand is the largest exporter of aromatic rice on the world market. Thus, the impact of the two rice 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 US$ 4.76 billion (Table 2), and of milled rice US$ 15.3 billion, and that of RD15 US$ 485.6 million (Table 3) for paddy and US$ 1.6 billion for milled rice. Thus, during 1989–98, the two varieties, RD6 and RD15 yielded 42.0 million tons paddy or 26.9 million tons milled rice worth US$ 16.9 billion.

In Vietnam, the breeders have taken the advantage of mutation techniques to rapidly develop improved varieties from local, well-adapted rice germplasm. Only six years after mutagenic treatment in 1993, two improved varieties, 'TNDB-100' and 'THDB', with early maturity and improved grain yield were released in the Mekong Delta. These varieties are tolerant to acid sulphate and/or saline soils. The varieties are grown on over 220,000 ha (Ro & At, 2000).

In Myanmar, the rice mutant variety 'Shwewartun' was developed and released in 1975 after seed irradiation of 'IR5' 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 1989–93, it covered annually more than 0.8 million ha – 17% of the 4.8 million ha area under rice in Myanmar (Myanmar Agricultural Service, Planning and Project Division, Ministry of Agriculture, Yangon, 1995).

*Barley*

The high-yielding and short-height mutant cultivars of barley 'Diamant' and 'Golden Promise' have made a major impact on the brewing industry in Europe. The mutants have also been used as parents of many

*Table 4.* Area and production of 'Golden Promise' barley mutant variety in Scotland*

| Year | Certified seed (t) | Area planted (ha) | Production (t) | Malting price (BPS) | Crop value (BPS) |
|---|---|---|---|---|---|
| 1977–78 | 8,390 | 19,200 | 86,400 | 84.10 | 7,266,240 |
| 1978–79 | 9,499 | 21,700 | 97,650 | 95.07 | 9,283,586 |
| 1979–80 | 11,163 | 25,500 | 114,750 | 97.45 | 11,182,388 |
| 1980–81 | 35,818 | 81,900 | 368,550 | 108.01 | 39,807,086 |
| 1981–82 | 32,150 | 73,500 | 330,750 | 119.46 | 39,511,395 |
| 1982–83 | 31,281 | 71,500 | 321,750 | 130.76 | 42,072,030 |
| 1983–84 | 30,914 | 70,600 | 317,700 | 125.34 | 39,820,518 |
| 1984–85 | 23,509 | 53,600 | 241,200 | 116.88 | 28,191,456 |
| 1985–86 | 14,408 | 32,800 | 147,600 | 121.82 | 17,980,632 |
| 1986–87 | 10,872 | 24,800 | 111,600 | 128.79 | 14,372,964 |
| 1987–88 | 7,831 | 17,900 | 80,550 | 127.15 | 10,241,933 |
| 1988–89 | 6,535 | 14,900 | 59,400 | 139.24 | 8,270,856 |
| 1989–90 | 5,772 | 13,200 | 59,400 | 129.40 | 7,686,360 |
| 1990–91 | 3,141 | 7,200 | 32,400 | 126.08 | 4,084,992 |
| 1991–92 | 789 | 1,800 | 8,100 | 128.04 | 1,037,124 |
| 1992–93 | 623 | 1,400 | 6,300 | 120.86 | 761,418 |
| 1993–94 | 404 | 900 | 4,050 | 124.21 | 503,051 |
| 1994–95 | 353 | 800 | 3,600 | 144.10 | 518,760 |
| 1995–96 | 769 | 1,800 | 8,100 | 124.42 | 1,007,802 |
| 1996–97 | 777 | 1,800 | 8,100 | 94.39 | 764,559 |
| 1997–98 | 453 | 1,000 | 4,500 | 94.01 | 423,045 |
| 1998–99 | 812 | 1,800 | 8,100 | 78.50 | 635,850 |
| 1999–00 | 644 | 1,500 | 6,750 | 76.53 | 516,578 |
| 2000–01 | 481 | 1,100 | 4,950 | 78.77 | 389,912 |

*/ Hall, G., 2001. Scottish Agricultural Science Agency, East Craigs, Edinburg, Scotland; BPS – British Pound Sterling.

leading barley cultivars. For example, more than 150 leading barley cultivars in several countries in Europe, North America and Asia were derived from crosses involving Diamant. The gamma-ray induced cultivar Diamant was officially released in Czechoslovakia in 1965. Diamant was 15 cm 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 was 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 (Bouma & Ohnoutka, 1991).

The cultivars Golden Promise and Diamant have added billions of dollars to the value of the brewing and malting industry. For example, during the 1960's and 70's, the cultivar Golden Promise was widely used by the brewing industry in the UK and Ireland for the production of beers and whisky. This cultivar had stiff straw, high yield and improved malting quality, and was produced by gamma ray irradiation of seeds of the well-known malting cultivar 'Maythorpe'. Even 30 years after its release, Golden Promise is still popular for its high quality in the production of premium quality ales and whisky by selected breweries in Scotland. Between 1977–2001, Golden Promise contributed US$ 417 million to grain production, primarily for brewing and malt in Scotland, as estimated from the sale of Certified Seed and based on average yield of 4.5 t/ha (G. Hall, pers. commun.). During the same period, Golden Promise was planted over an estimated area of 542,200 ha from Certified Seed. In addition, during 1977–2001, the Certified Seed of Golden Promise was worth US$ 86.8 million, based on an average price of US$ 366/t (Table 4). The added value from the production and sale of beer and whisky was several times more than that of the grain.

Recent studies have shown that Golden Promise is also salt-tolerant whereas the parent cultivar

Maythorpe is salt-sensitive. The only known genetic difference between the two lines is the *ari-e*GP mutation, which results in the semi-dwarf phenotype of Golden Promise. The expressed gene(s) induced by salinity were investigated using the mRNA differential display technique. Several genes related to salt tolerance were identified. Among them, one encoding a regulatory Leucine Zipper transcription factor was expressed in the absence of salt in Golden Promise but not in Maythorpe. This gene was over expressed in Maythorpe under high salinity but not in Golden Promise. In addition, a chloroplast gene *petB*, a vacuolar ATPase, a *BARE-1* copia-like retro-element and RuBisCo subunits were induced by salt in the salt-sensitive cultivar 'Maythorpe' (Forster, 2001; Wei et al., 2001).

*Wheat*

A durum wheat cultivar 'Creso' was developed in 1974 in Italy by crossing 'Cappelli' mutant 'Cp B144' and spread over a large area within a short period. Already in 1984 this mutant cultivar shared 53.3% of the market of certified wheat seeds in Italy and was grown on 400,000 ha. According to Scarascia-Mugnozza et al. (1993), the estimated additional grain yield contribution of Creso at an average of 0.9 t/ha was valued at US$ 180 million per year and US$ 1,800 million over a decade of its cultivation (Table 5). At least five other cultivars were derived by crossing Creso with others cultivars and have been extensively cultivated.

In Pakistan, three wheat cultivars were released in Sind Province, Pakistan. The mutant cultivar, 'Jauhar 78' derived from 'Nayab' after neutrons treatment had high yield, wide adaptability, amber grain, and resistance to shattering. The cultivar 'Soghat 90' derived with sodium azide treatment of 'Pavon' had high grain and biomass yield, high protein and lysine content and tolerance to leaf-rust. The cultivar 'Kiran 95,' developed from 'WL-711' with sodium azide treatment, had also high yield. The three cultivars contributed an estimated US$ 87.1 million between 1991–99 to the income of the farmers (Arain et al., 2000). Among these, the cultivar Kiran 95 was planted on over 30% of the area under wheat, and added US$ 47.5 million to farm income during the last five years.

The wheat cultivar 'Sharbati Sonora', a mutant of 'Sonora 64', released in 1967, had better acceptance for grain colour by the consumer in the early years of the 'Green Revolution' in India.

*Table 5.* Economic value of durum wheat mutant varieties in Italy*

| Year | Area (ha) | Production (t) | Percent of total area | Market value (Billion ItL) |
|---|---|---|---|---|
| 1976 | 77,250 | 262,600 | 4.6 | 47.3 |
| 1983 | 430,000 | 1,505,000 | 25.1 | 632.1 |

*/Bozzini et al., 1984.

*Grain legumes*

Pulse crops are an important and major source of protein for the vast population of Asia. Of the six major pulse crops (mungbean, urdbean, lentil, grass pea, rajmash and field pea) mungbean and urdbeans are cultivated on over 6 million ha in India, which is more than 25% of the area under all pulse crops (Dixit et al., 2000). In India, 8 mutant cultivars of mungbean (*Vigna radiata* L. Wilczek), 4 of urdbean (blackgram – *Vigna mungo* L. Hepper) and 3 of lentil (*Lens culinaris* Medik.) with high yielding capacity have contributed several million dollars to the country's agriculture.

*Mungbean*

In India, the release of early maturing cultivars resistant to Yellow Mosaic Virus (YMV) and their suitability to different cropping systems has resulted in an increased area and production in several states particularly, Bihar, Gujrat, Maharashtra, Rajasthan and Punjab. Early maturity made it possible to plant these cultivars during spring and summer after the harvest of potato, sugarcane, wheat and mustrad (Dixit et al., 2000). These cultivars include several mutant cultivars e.g. 'MUM 2', 'BM 4', 'LGG 407', 'LGG 450', 'Co4', 'Dhauli' (TT9E), 'Pant moong-1' and 'Tap-7'. Most of the mutant cultivars combine high yield and early maturity with tolerance or resistance to YMV. Mutant cultivar MUM 2, derived from 'K851' and released in 1992, is resistance to YMV, matures between 60–70 days, and yields on average 1.2 t/ha compared with the national average of 600 kg/ha and of 1.1 t/ha of the high yielding cultivars (Dixit et al., 2000).

In Pakistan, nine mutant cultivars of mungbean with induced early and uniform maturity, short stature and large seed size have been released. 'NIAB Mung 92' was approved in 1996 and 'NIAB Mung 98' in 1998 for cultivation in the Punjab province. Both cultivars are high yielding and resistant to YMV and *Cercospora* leaf spot. A major part of the 120,000 ha under mungbean in Pakistan is planted with these mutant cultivars (M.A. Awan, pers. commun.).

*Urdbean*

In urdbean (also known as blackgram), the early maturing cultivars have made it possible to grow them during spring under assured irrigation in the plains of North and Southern states of India. Several urdbean mutant cultivars have been released that include 'Vamban 2', 'TU 94-2', 'Co4', 'Sarla B-14–4', 'TAU-1', 'TAU-2', 'TPU-4', 'TAU-5' and 'TU-94-2'. These cultivars are widely grown and form almost 50% of the total breeder seed of urdbean in India. The Bhaba Atomic Research Center, Trombay, Mumbai released TAU-1, TAU-2, and TPU-4 that were developed through crosses with large seeded neutron induced mutants 'UM-196' and 'UM-201'. The mutants had 1000-grain weight of 56 and 69 g compared with 50 g of the parent cultivar 'No.55' (S.E. Pawar, pers. commun.). The cultivars Vamban 2 and Sarla B-14-4 were derived as mutants from cultivar 'T-9', which is susceptible to YMV. Both mutants are resistant to YMV, tolerant to drought, have early maturity of 70 days, and yield 0.82 t/ha (Dixit et al., 2000) compared with the average yield of 0.55 t/ha in India.

During 1989–99, the mutant cultivar TAU-1 covered more than 95% of the area under the crop in the State of Maharashtra, and contributed an estimated US$ 64.7 million from increased production of urdbean (Pawar, pers. commun.). It has been estimated that in the province Vidarbha alone the economic gain would amount to US$ 1.1 million (Rs 55,000,000) if TAU-1 contributed as little as 50 kg/ha additional production (Bhatia, 1991).

*Chickpea*

As a result of radiation-treatment of local cultivars and subsequent selection for disease resistance in segregating mutant populations, the first high yielding chickpea mutant cultivar 'CM-72' resistant to *Ascochyta* blight was released in Pakistan in 1983. Between 1988–90, in the North Western Frontier Province (NWFP) the chickpea yields were 44–45% higher than the average of the previous five years, mainly because of the cultivation of this excellent disease-resistant cultivar. In 1995, a new mutant cultivar 'CM-88' was released with multiple resistance (*Ascochyta* blight and *Fusarium* wilt) and recently 'CM-98', which is disease resistant and high yielding. The current area covered by these mutant cultivars is 350,000 ha, more than 30% of the total area under chickpea. The additional income to farmers has been estimated at US$ 9.6 million per year. More than 800 new disease resistant and morphological mutants have been produced to broaden the chickpea gene pool in the country (M.A. Awan, pers. commun.).

*Soybean*

In Vietnam, nearly 45% of the cultivars currently grown are from induced mutations. These include 'DT84', 'DT90', 'DT95', 'M103', 'V48' and 'A5' with improved yield, large grains and suitability for cultivation of 2–3 crops per year. Thus over the last 20 years, the cultivated area has increased by 228% and the output by 552% (Ro & At, 2000).

Eleven soybean cultivars were released by the Heilongjiang Academy of Agricultural Sciences, Harbin, China P.R., after mutagenic treatment with X-rays or gamma rays or by crossing mutants with other cultivars. The mutant cultivar 'Heinong 35' was released in 1990. Up to 1994, the planting area under this cultivar was 700,000 ha. Due to its high protein content, it was exported to Japan in large quantities. From 1967 onwards, 54 mutant cultivars of soybean were officially released in China with important mutant traits such as early maturity, improved yield, virus and disease resistance, lodging resistance, drought tolerance, improved protein and oil content, and hyper-nodulation (Wang, 1995).

In USA, soybean is a major crop and an important source for edible oil production. The *lx* loci determine shelf life and quality of soybean oil. Two groups of researchers in Japan used gamma irradiation to break the repulsion linkage between the loci *lx1* and *lx2* in one case and to induce a change in *LX1* in another case. These mutants have been used extensively to produce cultivars that are lipoxygenase free, and include among others mutant-derived cultivars 'IA2025', 'IA2027', 'IA2028', 'IA2029', 'IA2030', 'IA2032', and 'IA2033' released in Iowa. Pioneer Hi-Bred International has also used some of these mutants to release cultivars with altered oil composition (J. Fehr, pers. commun.).

*Common bean*

In common bean radiation-induced early bush type mutants of the cultivar 'Michelite' and 'NEP-2' (white-seed coat mutant) and their derivatives were widely used in the pedigrees of many white-seeded bean cultivars in North America. During the 1970 and 1980's most of the white-seeded bean cultivation area in Michigan was covered by bush mutant derivatives.

Currently, 40% of the 300,00 acres of white beans in Michigan are under cultivars derived from Michelite and NEP-2 (Nichterlein, 1999).

*Pea*

In pea, a gamma ray induced mutant cultivar 'Wasata' with tendrils instead of leaves (*afila*) was released in Poland in 1979. This cultivar was further used for breeding to produce the cultivar 'Sum' with short height. An *afila* type cultivar 'Piast' was released from the cross Sum × Metzer in Poland in 1995 (R. Madajewski, pers. commun.). The cultivar 'Piast' is a yellow seeded dry pea that combines high yield with good standing power and has been released in Great Britain in 1998 as 'Ramrod' (United Oilseeds, 1997). In the *afila* type cultivars, the leaflets are converted into tendrils but stipule leaves are still present. The plant habit leads to plant architecture that allows good aeration, better light penetration and improved colour. The upright crop canopy gives high standing power that allows harvesting without choking the combines. It should be mentioned here that several semi-leafless pea cultivars that have derived from spontaneous *afila* mutants are also grown widely in Canada and other countries.

*Oil and industrial seed crops*

Perhaps the greatest global impact of plant breeding in oil seed crops has been in rapeseed and sunflower in which spontaneous and induced mutants have been used in combination with conventional breeding methods to modify oil composition and increase yield.

*Rapeseed – canola (double-zero rapeseed)*

Rapeseed has high erucic acid content in oil and high glucosinolates in meal. Both erucic acid and glucosinolates are nutritionally undesirable. Breeders have reduced the amount of erucic acid and gulcosinolates and have developed canola. Canola quality is derived from three *Brassica* species: *Brassica rapa* L. (syn. *Brassica campestris* L.) also known as turnip rape or Polish rapeseed, *Brassica napus* L. and *Brassica juncea* (Indian mustard). Canola cultivars (also known as 'double zero', 'double low' or '00' cultivars) have less than 2% erucic acid in oil and less than 30 $\mu$m/g of aliphatic glucosinolates in the meal. Continued research efforts have reduced the levels of erucic acid to less than one percent and glucosinolates to less than 20 $\mu$m/g.

The low erucic acid content of rapeseed *B. napus* ('0' quality) originates from a spontaneous low erucic mutant of the German forage rapeseed cultivar 'Liho' (*B. napus*), which was discovered in Canada when searching the world's germplasm for its oil quality. The spontaneous mutation blocked the biosynthesis of eicosenoic and erucic acids. The result was moderately high oleic oil with low erucic acid content. The mutant was used to develop low erucic rapeseed cultivars 'Oro' (*B. napus*) in 1968 and 'Span' (*B. rapa*) in 1971 (Downey, 1990). While the rapeseed oil quality was bred into suitable cultivars, efforts were made by breeders to improve the meal quality by reducing the antinutritional glucosinolates. The trait for low glucosinolate content of the meal was discovered in germplasm of the Polish summer *B. napus* cultivar 'Bronowski' in 1967, which was utilized to develop low erucic, low glucosinolate ('00') cultivars. Similarly, canola quality was developed in *B. rapa* (Canola Connection, 2001) and recently in *B. campestris* using spontaneous mutants and intra- and inter-specific hybridization (Potts et al., 1999)

The expansion of canola in Canada and Europe is primarily due to its modified fatty acid composition, i.e. low erucic acid, and high oleic acid and the low content of glucosinolates, which improved the nutritional quality of the oil for human nutrition and meal for livestock feed (Agriculture and Agri-Food Canada, 1999). Plant breeders also recognized that by changing other fatty acids, different nutrient and processing characteristics could be produced in rapeseed oil, and that mutation techniques could be utilized to further modify the fatty acid composition. The first summer canola cultivar (*B. napus*) of this category was registered in Canada in 1987 under the name 'Stellar'. Stellar has significantly reduced linolenic acid content in the oil (3%). Linolenic acid causes rancidity of oil, its reduction in canola leads to a better processing and keeping quality of the oil. The low linolenic trait traces back to Germany, where mutants ('M57', 'M3', 'M6', 'M8', 'M11') with reduced linolenic acid content were selected after chemical mutagenesis of the Canadian low erucic acid cultivar Oro (*B. napus,* 10% linolenic acid). The mutant M57 with 5.6% linolenic acid was mutagenized again, and a double mutant 'M47' was selected for reduced (up to 3.3%) linolenic acid (Röbbelen, 1990). The Oro mutants were utilized in breeding programmes in Canada, Australia and Europe as a source of low linolenic acid not only for *B. napus*, but also for other *Brassica* species. In Sweden, they were crossed with winter *B. campestris* to develop low linolenic Canola. In Australia, they were crossed with hybrids between *B. napus* and *B. juncea.* As a result, low linolenic *Brassica* cultivars such as Stellar, 'Apollo' in Canada, 'Nzelenic' in Australia were released with oil of good storage and frying characters (Wong & Swanson, 1991). The low linolenic trait of the cultivar Stellar is relatively stable over wide environments. Genetic studies using Stellar have shown that two major genes, *L1* and *L2*, control low linolenic acid. A desaturase gene *fad3* is linked to *L1* (Scarth & McVetty, 1999).

A further modification of canola oil was achieved by using seed and microspore mutagenesis. For spring canola, radiation treatment of seed was applied and $M_5$ lines of the cultivar 'Regent' were selected with increased oleic acid content from 63 to 79%, and reduced linoleic content from 20 to 7%. The selected $M_5$ mutants were then used for developing breeding lines with 85% oleic acid spring canola. For winter canola, chemical microspore mutagenesis and doubled haploid technique were combined and two winter canola mutants with high (79%) oleic acid content of the cultivar 'Winfield' were isolated. The high oleic acid canola also has lower levels of saturated palmitic and stearic acids (6%) compared with that of 16% in olive oil (Wong & Swanson, 1991), thus it has the additional nutritional value to the consumers. Compared to sunflower and olive, oilseed rape has a much wider climatic adaptation and can be grown in many parts of the world. As a result of these breeding activities, at least 24 new registered cultivars of *B. napus* with modified fatty acid composition have been developed in Canada that directly or indirectly descend from induced mutations. The high oleic and/or low linolenic acid traits of these cultivars have been derived solely through induced mutagenesis. These include 'Allons', Apollo (University of Manitoba), '46A40', '46A41' (Pioneer Hi-Bred International), 'IMC02', 'IMC03', 'IMC104', 'IMC105', 'IMC106RR', 'IMC130', 'IMC140', 'IMC201', 'IMC202', 'IMC203RR', 'IMC204', 'IMC205', 'IMC302', 'IMC303' (InterMountain Canola), 'Nex 700', 'Nex 705', 'Nex 710', 'Nex 715', 'Nex 720' (DowAgroSciences) and 'LG3930' (Limagrain) (G. Rakow & M. Hartman, pers. commun.).

Some of the new canola (*B. napus*) lines descend from the combination of two induced mutant traits 'high oleic' and 'low linolenic' as shown for the canola lines '45A37' and 46A40. They have been developed through the combination of induced mutagenesis of cultivars 'Regent', 'Topas' and 'Andor' and crossing with low linolenic canolas Stellar and Apollo to obtain high oleic acid trait. The processed oil of these lines has even higher levels of oleic acid than that of peanut and olive. It is considered that the induced mutation in these lines is similar to that in *fad2* mutants of *Arabidopsis thaliana* (Health Canada, 1999).

Microspore mutagenesis has also been used in combination with *in vitro* screening for the development of *B. napus* tolerant to the herbicides imidazoline and chlorosulfuron (Swanson et al., 1988). A further development has been the cloning of acetohydroxyacid synthase (*AHAS*) genes from microspore mutagenesis and their transgenic transfer into canola for breeding herbicide resistant cultivars. Plants with these mutated genes confer a high level of tolerance to sulfonylureas (e.g. chlorsulforon) and imidazolinones widely used as herbicides. The primary action site of these herbicides is the enzyme *AHAS* (Huang, 1992).

The contribution of canola cultivars to the Canadian economy has been outstanding (G. Leblanc, pers. commun.). Canola is Canada's third most important grain export, after wheat and barley. During 2000, Canada planted 5,564,000 ha under canola, and harvested 4,815,900 ha with production of 7,118,700 tons seed, with exports of canola oil of 869,932 tons valued at US$ 350.5 million (Canadian Grain Commission, 2000). During 1995–99, Canada exported 165.7 million tons of rapeseed oil, valued at US$ 4,745 million (FAO, 2001).

The germplasm with the desired oil composition has spread widely among the plant breeders and the benefits reaped have been global. It is hard to partition the specific contribution of the spontaneous mutants and induced variation from that of recombination-based breeding. Of course, not all canola oil production and exports can be attributed to mutated germplasm. However, in Canada, Australia, and Europe, many cultivars with modified oil-profile are based on mutant germplasm.

*Sunflower*

Sunflower oil is comprised primarily of palmitic, stearic, oleic and linoleic acids, with oleic and linoleic accounting for about 90% of the total fatty acid content in the conventional oil. It has been recognized that there is an inverse relationship between oleic and linoleic acid, which is highly influenced by environment, especially temperature during the growing season. Under the cool northern climates, sunflower seed has high linoleic acid-content in contrast to high oleic acid of seed produced under warmer southern areas. While a high linoleic acid concentration is desirable in sunflower oils used in soft margarines and salad dressings, high oleic acid content is preferred for many other applications, since oleic acid is oxidatively more stable than linoleic acid. As a consequence, oxidative stability of conventional crude sunflower oil derived from seed grown in southern climates is nearly twice that of crude oil extracted from northern-grown seed.

The open-pollinated mutant cultivar 'Pervenets' with modified oil composition was developed after chemical mutagenesis with dimethylsulphate in Krasnodar, Russia (the former USSR). It was the first sunflower cultivar with high oleic acid and low linolenic acid oil (Soldatov, 1976). The partially dominant allele *Ol* controls the high oleic acid mutant trait (Miller & Fick, 1997). Moreover, in Pervenets, an increase in oleic acid (from 64% to 79%) during seed formation and ripening was observed in conjunction with a decrease in linoleic acid (from 26% to 15%).

An important development in sunflower breeding was the introduction of a high oleic acid mutant into the USA. After 1980, the Pervenets germplasm became available to sunflower breeders in the USA and Europe, where it has been used widely as the primary source of breeding hybrid cultivars with high oleic acid (Skoric, 1988). Oil with high oleic acid content has steadily gained market acceptance, especially for food and industrial purposes where high oxidative stability is required (Fick & Miller, 1997). A large number of hybrid cultivars sold under the generic name 'NuSun' trace their pedigree to the Pervenets genes for high oleic acid. During 1999, 450,000 ha were planted with these cultivars in USA, and the expected area in 2000 was one million hectares (Rampton, 2000) that would yield approx. 270,000 t oil worth US$ 149.3 million at the average export price of US$553/t during 1999. Male sterility of some lines used for hybrid seed production in sunflower was also obtained through mutations induced with mitomycin C and streptomycin (Jan & Rutger, 1988; Jan, 1992).

*Linola*

Linseed or flax (*Linum usitatissimum* L.) is grown either for the oil (linseed) extracted from the seed or for fiber (flax) from the stem. Linseed oil is unique among other vegetable oils in that it contains more than 50% linolenic acid; the 18-carbon fatty acid with three double bonds. The double bonds rapidly react with oxygen in the air to polymerize into a relatively soft and flexible film. As a result the oil has its greatest use in industrial product applications such as paints, solvents, inks, soaps and linoleum flooring. The most recent modification of oil composition with induced mutations has been the development and release in Australia and Canada of linseed cultivars of 'linola' type with oil cooking quality as an alternative crop in rotation with wheat (Green, 1986; Dribnenki et al., 1996). The Division of Plant Industry, CSIRO, Canberra, transformed linseed oil into edible oil by reducing linolenic and raising linoleic acid levels similar to that in traditional sunflower oil. Three new linseed cultivars 'Wallaga', 'Eyre' and 'Argyle' have been released under the generic types called Linola® and are grown in Australia and New Zealand. Linola cultivars have golden yellow seeds. Eyre is derived from an $F_8$ bulk originating from a single-plant selection taken in the $F_4$ of the cross 'Glenelg'/CPI 84495//4*'Zero'. 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 (Green, 1986). An agreement between the Canadian and Australian breeders led to work on linola cultivars for Western Canada. Subsequently, low linolenic linseed cultivars were registered in Canada under 'Linola 947', 'Linola 989' and 'Linola 1084'. In 1995, linola production was 12,000 t in Australia, 100,000 t in Canada and 250 t in the UK, the latter mainly for seed production (Green & Dribnenki, 1996). In 1997, farmers in Western Canada grew 750,000 ha of linseed and 70,000 ha of linola (Domier, 1997).

*Groundnut*

The release of 'TG' cultivars of peanut in India has contributed many million dollars to the Indian economy (G.S. Murty, pers. commun.). A recently released peanut mutant cultivar 'TG-26', developed at Bhabha Atomic Research Center, Bombay, India yielded 4,6 t/ha nuts, an increase of 6.4–40.9% over the checks (Kale et al., 1997). In China, 14.7% of the new peanut cultivars were produced through the direct use of induced mutants or by the use of mutants in cross breeding. The cumulative cultivated area of these 33 mutant cultivars accounts for 19.5% of the total area under peanut in China (Qiu et al., 1997).

*Cotton*

A gamma ray induced, high yielding mutant cultivar of cotton 'NIAB-78' was released in 1983, and was grown mostly in Punjab, Pakistan. Developed at the National Institute of Agricultural Botany, NIAB-78 had a marked influence on sustaining the textile industry of Pakistan, and contributed to its economy in several ways. The cultivar has a shorter stature, determinate growth habit, tolerance to heat, and escaped bollworm attack due to its early maturity. Its early maturity made it an ideal cultivar in the cotton-wheat rotation. Within five years of release, its cultivation contributed enormously to the growth of the textile industry in the country and doubled cotton production in Pakistan. NIAB-78 covered about 70.8% of the total cotton area in Punjab in 1988. It is estimated that during the ten years following its release, it contributed more than US$ 3.0 billion in cotton production, and saved the textile industry of Pakistan that was threatened by reduction in cotton production from insect pests. The added income to cotton growers from the use of NIAB-78 in Pakistan from 1983 onwards has been estimated at US$ 486 million (M.A. Awan, pers. commun.). Its production value for the year 1999–2000 was estimated at US$ 38.4 million (M.A. Awan, pers. commun.). During 1999–2000, NIAB-78 was planted over more than 90% of the area under cotton in the Province of Sind, Pakistan. It has proven to be a remarkable cultivar in its wide adaptability and tolerance to stress. When boll shedding occurs under extreme heat stress, it can produce a second flush of flowers. Even after 14 years of its first release, nearly 25% of the area under cotton in Pakistan is planted to this cultivar.

The new mutant cultivar 'NIAB Karishma' released in 1996, and derived from a cross of the mutant cultivar NIAB-86 with an American strain 'W 83–29', is early maturing, has improved heat tolerance, and high yield potential. NIAB Karishma has been cultivated on 486,000 ha and brought farmers US $ 17 millions income (M.A. Awan, pers. commun.)

In China, the cotton cultivar 'Lumian No. 1' derived from gamma ray treatment was released in 1974. This high yielding cultivar's annual cultivation area exceeded 1 million hectares until the late 80's (Maluszynski et al., 1995). It had been one of the most widely grown cotton cultivars in China.

*Non-narcotic poppies*

Scientists at the Central Institute of Medicinal and Aromatic Plants (CIMAP) in India have developed the alkaloid-free opium-less poppy (*Papaver somniferum* L.) cultivar 'Sujata'. Occasionally breeders identify opium-less poppy plants, but their progeny have normal levels of opium. Breeders irradiated seed with 15 Gy gamma rays and found one plant containing no opium. The current cultivar is now in its seventh generation as an experimental crop. The cultivar might result in a non-narcotic version of a seed crop that is high in fatty acids. Poppy oil has a long history as a food product in Asia Minor, with India currently importing 1,000 t of poppy oil per year (Stokes, 2001). Such a cultivar and its derivatives would allow release of non-narcotic cultivars of poppy. The existing poppy cultivars produce opium and the illicit trade of opium and poppy husk is a major global concern of developed and developing countries alike. The non-narcotic poppy cultivars would give the growers a high value crop for poppy seed and oil production without problems of drug control.

*Vegetatively propagated plants*

In the vegetatively propagated species, the entries in the mutant cultivar database are limited to only 97 out of 1700 crop cultivars (Maluszynski et al., 2000). Despite the small number, some of them have made considerable economic impact.

*Peppermint*

In peppermint, *Mentha piperita* L., two cultivars 'Todd's Mitcham' and 'Murray Mitcham' with tolerance to *Verticillium* wilt were released in 1971 and 1976, respectively. These cultivars were developed after neutron and X-ray treatment of stolons of the wilt-susceptible cultivar 'Mitcham' followed by extensive screening in infested fields (Murray & Todd, 1972). These two mutants are among the three cultivars recommended for controlling *Verticillium* wilt in USA, where 90% of the world's peppermint oil is produced (Foster et al., 2000).

The global production of mint oil is estimated at around 5,000 t with 5% annual increase in consumption. Currently Todd's Mitcham forms the bulk of the world's production of mint oil (Peterson & Bienvenu, 1998). Based on average bulk sale price of US$ 50/kg, the current production of 4,000 t of mint oil in USA is worth US$ 200 million. After packaging, mint oil is sold for US$ 27.92 per 120 ml bottle on the global market. Based on this price, its current market value would be approximately US$ 930 million.

*Fruits*

In fruit trees, 48 mutant cultivars have been listed in the FAO/IAEA Database 2000. Among these are mutant cultivars of orange (mandarins) (5), fig (1), apple (10), banana (2), apricot (1), sweet cherry (8), sour cherry (4), peach (2), pomegranate (2), pear (5), Japanese pear (2), and one cultivar each of black currant, ribes, raspberry and grape.

*Table 6.* Origin and characters of Japanese pear mutant cultivars (Amano, 2001-modified)

| Parent | Mutant | Blackspot disease | Compatibility |
|---|---|---|---|
| Nijisseiki | | Susceptible | Self incompatible |
| | Gold-Nijisseiki | Resistant* | Self incompatible |
| | Osa-Nijisseiki | Susceptible | Self compatible** |
| Osa-Nijisseiki | | Susceptible | Self compatible |
| | Osa-Gold | Resistant* | Self compatible |
| Shinsui | | Susceptible | Self incompatible |
| | Kotobuki-Shinsui | Resistant* | Self incompatible |

* Gamma radiation induced.
** Spontaneous mutation discovered by a farmer.

*Table 7.* Economic contribution of mutant varieties in Japan for 1997*

| Crop | Value (million US$) |
|---|---|
| Rice (18 cultivars) | 937.0 |
| Pear (3 cultivars) | 30.0 |
| Soybean (4 cultivars) | 5.0 |
| Peach, chrysanthemum | 1.2 |
| Total mutant varieties | 973.2 |

*/Anonymous, 2000.

'Ruby Red', the first commercial grapefruit cultivar with red-pigmented fruit and blushed peel, originated from a spontaneous mutation discovered in Texas in 1929. However, its flesh color faded as the harvest season progressed, and the juice colour was not accepted. A redder grapefruit was desired. Seeds and bud wood were irradiated and two cultivars were obtained. 'Star Ruby', released in Texas in 1970, was obtained by thermal neutron irradiation of 'Hudson' seeds. It is seedless, red flesh and has gained wide acceptance, but its yield is variable. 'Rio Red', released in 1984 in Texas, was derived from irradiated bud wood of Star Ruby with thermal neutrons (Hensz, 1991). It has red flesh and a good yield (Spiegel-Roy, 1990). Rio Red became the preferred cultivar in Texas and in other grapefruit growing areas. The fruits of both cultivars are sold under the trademark 'Rio Star'. In Texas, Rio Star grapefruit is currently grown on 7,300 ha, which is 75% of the grapefruit production area in Texas (Sauls, 1999). The development of the two radiation induced mutant cultivars is considered as the most significant break through in grapefruit growing in Texas since the discovery of Ruby Red in 1929.

The economic impact of the cultivar 'Gold Nijisseiki' of Japanese pear (*Pyrus pyrifori*a) is well documented. This cultivar was developed with chronic radiation in a gamma field, Ohmiya, Ibaraki. It is more resistant to black spot disease (caused by *Alternaria alternata*) than its parent 'Nijisseiki'. The parent cultivar was susceptible to blackspot disease. The additional annual income by growing this cultivar is almost US$ 30 million according to the calculations of a farmers' group cooperative (Anonymous 2000; E. Amano, pers. commun.). New mutant-cultivars 'Osa-Nijisseiki' and 'Osa-Gold' with improved disease resistance have been released recently (Table 6). These mutants are also self-compatible and eliminate the need of planting pollinators. In 1997, the over all economic contribution of mutants of rice, pear, soybean and peach in Japan has been estimated at US$ 973.2 million (Table 7).

*Ornamental plants*

Before the development of *in vitro* techniques, many mutants in ornamentals, e.g. achimenes, chrysanthemum, carnation, roses and streptocarpus, were obtained by irradiating rooted stem cuttings, detached leaves, and dormant plants (Broertjes & van Harten, 1988). The altered flower colour and shape, growth-habit (dwarf or trailing) and other novel phenotypes of commercial value were selected. According to the FAO/IAEA Database, of the 552 mutant cultivars of floricultural plants, most were in chrysanthemum (232), followed by alstroemeria (35), dahlia (36), bougainvillea (12), rose (61), achimenes (8), begonia (25), carnation (18), streptocarpus (30), and azalea (15) (Maluszynski et al., 2000). Since the effect of mutation in ornamentals is very visible, selection for changed flower colour, shape, and size is easy, and almost anything, which is novel, is of value. Hence, mutation techniques have become a major tool for breeding ornamental plants (Maluszynski et al., 1995).

The exact number of ornamental cultivars released and their value is difficult to estimate. Commercial companies often do not report the origin of the induced mutant cultivars of ornamentals and their value is kept a trade secret. In many developing countries, the cultivars are registered but no records are maintained on their spread, because cultivars can be multiplied freely without notifying the breeder. For example, the National Botanic Research Institute (NBRI), Lucknow, India, during the past 17 years released 70 mutation-induced cultivars of ornamentals – chrysanthemum (42), rose (12), bougainvillea (4), lantana (3), hibiscus (1) and portulaca (6) (S.K. Datta, pers. commun.). Similarly, the Department of Applied Radiation and Isotopes, Kasetsart University, Thailand released six mutant cultivars of cannas, fifteen of chrysanthemum and two of portulaca. No data are available on the spread of these new cultivars. The material generated by plant breeders is handed over to the growers free of charge for further multiplication (Srinut Lamseejan, pers. commun.).

*Chrysanthemum*

Chrysanthemum (*Chrysanthemum* spp. or *Dendranthema* spp.) is one of the most widely cultivated ornamentals, and is grown for the cut flower and potted plant markets. Because it is propagated vegetatively, mutations are most important for breeding new cultivars. Some 210 mutant cultivars have been registered in the FAO/IAEA Database in this species and more are being produced in different countries, mainly in Germany, India, Japan, and the Netherlands. The most desired characteristics for customers are flower colour, flower shape and shelf life. Many mutants have been developed and released with changes in flower colour. The general practice has been to select cultivars with pink flowers from which red, white and yellow mutants are obtained via irradiation of the cuttings (Broerties & van Harten, 1988). Recurrent irradiation was used in several cases to develop chrysanthemum cultivars, e.g. recurrent irradiation with X-rays starting from the parent cultivar ‘Horim’ resulted in 8 new released cultivars with different flower colours. Induced mutations were used also to modify more complex characters. Research done in Germany and The Netherlands resulted in the development of cold tolerant and early flowering chrysanthemum mutants (Huitema et al., 1991). These mutants obtained with X-rays facilitated more economical production of this plant under European winter conditions.

For a long time chrysanthemum has been – after roses – the second most important cut flower in the Netherlands. It covers about 20% of the total annual turnover at the national flower auction. Chrysanthemum breeding in The Netherlands is completely in the hands of private enterprise. Three major breeding companies release most new cultivars, whereas some ten additional smaller companies also breed chrysanthemum. The share of mutant or mutant-derived chrysanthemum cultivars of the total flower market has been estimated at 30–40% during the last 5 years. The Dutch Varieties List for 1994 contained 42 recommended chrysanthemum cultivars of which some 23 were either mutants or derived from mutants. However, this cultivar list does not make any distinction between spontaneous mutants (‘sport’) and induced mutants. Breeders, for commercial reasons, do not reveal this type of information. According to the Flower Council of Holland, the total value represented by chrysanthemum at the flower auction in 1992 and 1993 amounted to 563 and 581 million Dutch guilders (about US$ 300–350 million), respectively. For example a series of mutants were derived from the officially registered cultivar ‘Reagan’ (1991). Twenty of these mutants (e.g. ‘Reagan White’, ‘Reagan Sunny’, Reagan Dark Splendid’, ‘Reagan Yellow’, ‘Reagan Salmon’ and ‘Reagan Orange’) appeared in the cultivar list in 1994 and represented 35–40% of the total Dutch flower market in 1992 and 1993. They were still represented in the ten best sold chrysanthemum cultivars at the national flower auction in 1994 and 1995 (A. Ashri and L. van Zanten, pers. commun.).

*Recent trends on induced mutations and mutation-derived cultivars*

From the present survey, it is clearly established that the plant cultivars derived from induced mutations have contributed billions of dollars to the economies of many countries. The main beneficiaries have been not only developing countries (e.g. India, China, and Pakistan), but also North American and European countries have gained from the release of mutant cultivars. Whereas the emphasis in the developing countries has been on food crops such as rice, North America and Europe have used mutants to improve crops for the processing industry e.g., edible oils from sunflower, rapeseed, and linseed, juice quality of grapefruit, essential oil from mint, and barley for brewing and malting industry.

The mutation of a gene that leads to the improvement of traits in a well-adapted existing cultivar or the derivation of a new cultivar from a mutant has currently been the basis of induced germplasm enhancement technology. In determining the value of a derived mutant cultivar, based on the area planted to the cultivar or from the value of the crop produced or processed into a product, it must be recognized that this value includes the contribution of many other genes introduced with recombination-based breeding as well as agronomic inputs, costs of packaging and processing. The mutant genes have added a significant part of this value. However, in many cases, the mutated gene has been the primary trigger in enhancing the value of a cultivar or a new crop. The transfer of some mutated genes created synergistic effects far beyond the changed mutant trait. For example, the changed oil profile of sunflower and rapeseed has led to a massive increase in the area planted to these crops.

Another outstanding contribution and unique feature of induced mutants in the breeding of several seed-propagated crops (e.g. barley, rice, sunflower and rapeseed) has been the global free access of the mutant germplasm and cultivars with transnational impact. For example, the dwarf genes induced in 'Calrose' in California were extensively used for rice breeding in Australia and Egypt. The high oleic acid mutants of sunflower induced in Russia (ex USSR) were widely used in the United States and Europe; those of rapeseed for low linolenic acid induced in Germany and high oleic acid induced in Canada have been used for breeding rapeseed in Australia, Canada and Europe.

The *denso* gene of barley mutant cultivar 'Diamant' was freely used for breeding more than 150 leading barley cultivars all over the world. The recent trend in some countries that allows patenting of plant genes and restricts the free access to genes and germplasm could be the biggest folly of plant breeding. It will kill the global synergy generated by the transfer of selected genes in local cultivars. Such patent laws will restrict access to new and novel genes whether mutated or molecularly manipulated, and will be a disaster for the economies of developing countries.

Presently, numerous mutants induced with chemicals, radiation and insertion of transposons or T-DNA are being used to produce saturated genetic maps and explore genomics, gene expression and gene regulation. The sequencing of some plant genomes are completed but assigning functions to many of the DNA sequences would not be possible but for the mutants induced with gamma rays, fast neutrons or chemical mutagens (Ahloowalia & Maluszynski, 2001). Thus, many mutants, which currently are of no breeding value, are becoming important tools in genome research. Unfortunately, thousands of such mutants were discarded and thrown away by geneticists and breeders, as something unwanted.

Progress in sequencing of rice and *Arabidopsis* genomes and expansion of many sequence databanks for numerous other species rapidly stimulates the use of reverse genetic approach for discovery of gene function, and in more general terms, for functional genomics. As sensitive mutation detection methods in DNA have been also established, the screening for DNA damages of particular gene sequence in pooled samples of various size from large mutated populations has become feasible. The first application of this strategy in plants has been called 'TILLING' targets induced local lesions in genomes (McCallum et al., 2000a). This is a new reverse genetic approach that combines high frequency of point mutations induced by classical mutation techniques with possibilities of detecting heteroduplexes between wild-type and mutant-DNA fragments using DHPLC (denaturing high performance liquid chromatography). High density of point mutations is a necessary condition for the use of this approach. Ionizing radiation and highly efficient chemical mutagens are usually applied for the development of mutated generations. This method was first demonstrated in *Arabidopsis* but was easily adopted for other plant species (McCallum et al., 2000b). Contrary to other knockout techniques, this procedure is not limited by the efficiency of transformation or activity of transposons. TILLING can be applied when the sequence of the targeted gene is known and the methodology for detection of single nucleotide substitutions is available. Besides DHPLC, other possibilities for heteroduplex analysis have been reported such as denaturing gradient gel electrophoresis (DGGE – Hauser et al., 1998; Temesgen et al., 2001), temperature gradient gel electrophoresis (TGGE – Matousek et al., 2000), or cleavage by the specific endonuclease (Oleykowski et al., 1998). Based on the analysis of the frequency of appearance of AFLP bands the frequencies of 1 mutation per 50,000 bp to 1 per 450,000 bp (5,500–50,000 mutations per mutant genome) were found in barley after mutagenic treatment with various mutagens (Caldwell et al., 2003). These investigation methods are still being improved and the

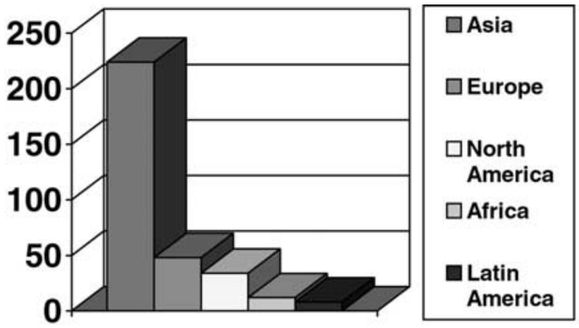


*Figure 1.* Number of mutant cultivars released since 1991.

entire strategy has been called 'mutation grid for plant functional genomics'.

In plant breeding projects, selection of the desired genotype is critical irrespective of the procedure used to create variation. The development of molecular probes offers a tremendous opportunity to select desired mutants. Hence, mutation induction, molecular marking of useful and selected mutations, sequencing of the mutated genes and the development of molecular probes will be critical in the continued and expanded use of induced mutations, mutants and cultivars. Particularly, mutants that modify the oil, starch, and protein quality will become increasingly important for breeding designer cultivars for industrial processing. Induced mutations will also play an increasing role in creating crop cultivars with traits such as enhanced uptake of specific metals, deeper rooting system, tolerance to drought and salinity, and resistance to diseases and pests as a major component of the environmentally sustainable agriculture.

The database on released mutant cultivars since 1991 shows specific trends of the activity on radiation induced mutations in over 30 countries. Of the 326 cultivars released, the largest number has been in China (117), followed by USA (28), Russia and Iraq (23 each), India (19), Bangladesh and Pakistan (14 each), Vietnam (11), Poland (10), Japan and Korea (9 each), Mali (8), Canada (6) and Brazil (5). Other countries released between 1 to 3 mutant cultivars each – Egypt, Estonia, Turkey (3 each), two each in Bulgaria, Costa Rica, Czech Republic, Mongolia and Sri Lanka, one each in Australia, Austria, Estonia, Germany, Ghana, Indonesia, Italy, Malaysia, Peru, Romania, Thailand and Ukraine. On a regional basis, more than 2/3 (68.4%) of the mutant cultivars have been released in Asia (224), followed by Europe 14.7% (48), North and Latin America 12.9% (42), and Africa 3.7% (12) (Maluszynski et al., 2000) (Figure 1).

To some extent, the data reflects the regional activity on the use of induced mutations to complement plant breeding. Although, the data in Africa is limited to only two countries, Mali and Ghana, the two crops, sorghum and cassava are critical to the food security in the two countries. On a crop basis, the maximum released mutant cultivars have been in rice, the most important food crop in the world. Thus, the released mutant cultivars are already a part of the over all strategy and commitment of the Joint FAO/IAEA Division to contribute towards the global food security, particularly in Asia and Africa, where maximum increase in population will take place during the next 20 years.

Our recent survey on the release of mutants has shown that there is a continuing trend in the use of mutations to derive improved cultivars. The survey (data not presented) also showed that the spread of a new mutant cultivar takes more than 5 years. The value and impact of new mutant cultivars thus need to be monitored at least after 5–7 years of their release.

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