



Role of Mutation Breeding in Crop Improvement- Past, Present and Future

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARJA/2016/29334

Editor(s):

- (1) Martha Isabel Torres-Moran, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Mexico.
(2) Rusu Teodor, Department of Technical and Soil Sciences, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.

Reviewers:

- (1) Ati Hassana Maryam, Federal University Dutsinma, Nigeria.
(2) João Carlos da Silva Dias, University of Lisbon, Portugal.
(3) Sunil Kumar Yadav, Jawaharlal Nehru Agricultural University, India.
Complete Peer review History: <http://www.sciencedomain.org/review-history/16970>

Review Article

Received 3rd September 2016
Accepted 10th November 2016
Published 19th November 2016

ABSTRACT

With the inevitable risk posed by global climate change to crop yield and ever increasing demands of agricultural production, crop improvement techniques have to be more precise in developing smart crop varieties. This review reviews the past, current progress and assesses the future directions in mutation breeding for crop improvement. It provides a background to plant mutation breeding strategies, basic and advanced techniques, and provides a critical review of this approach in comparison to other methods for the genetic improvement of crops. Mutation breeding is a fundamental and highly successful tool in the global efforts of agriculture to feed an ever increasing and nutritionally demanding human population. The physical and chemical mutagens, their effects and their utility are discussed. The induction of mutations has been used to enhance the yield, better nutritional quality and wider adaptability of world's most important crops such as wheat, rice, pulses, millets and oilseeds. The total area covered by commercially released mutant cultivars clearly indicates that they have played a significant role in solving food and nutritional security problems in many countries. Of all the mutant varieties developed, majority of mutants were produced through direct mutagenesis of the plant propagules, and also

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there are several reports of mutants derived by irradiating rooted stem cuttings, which paves the way for *in vitro* mutagenesis. The production of mutants by irradiation of *in vitro* cultured tissues provides a means to treat large populations which would not have been possible before. The accessibility of genomics information in the public domain combined with the recent advances in molecular biology techniques have paved the way for transforming old mutation techniques into the state of art technology for crop improvement and basic genomic research. The molecular tagging and molecular marker based identification shall bring new dimensions in gene technology. These would finally lead to rapid enhancement of crops with improved yield, increased biotic and abiotic stress and reduced agronomic inputs. Thus mutation assisted plant breeding will play a crucial role in the generation of designer crop varieties to address the threats of global climate change and challenges of world food insecurity.

Keywords: *Induced mutation; in vitro mutagenesis; molecular markers; genetic variability; developmental mutants.*

1. INTRODUCTION

Ever since the epoch-making discoveries made by Muller and Stadler eighty years ago, the application of mutation techniques by using different agents of physical and chemical nature has generated a vast amount of genetic variability and has played a significant role in modern plant breeding and genetic studies. The use of induced mutations over the past five decades has played a major role in the development of smart crop varieties all over the world. The widespread use of induced mutants in plant breeding programme across the globe has led to the official release of 3222 plant mutant varieties from 170 different plant species in more than 60 countries throughout the world [1] the developed varieties increase biodiversity and provide breeding material for conventional plant breeding thus directly contributing to the conservation and use of plant genetic resource.

The concept of induced mutagenesis for crop improvement developed dated back to the beginning of 20th century. During the past 80 years, mutation breeding has been successfully utilised for the improvement of crops as well as to supplement the efforts made using traditional methods of plant breeding [2]. Induced mutation is the ultimate source to alter the genetics of crop plants that may be difficult to bring through cross breeding and other breeding procedures [3]. Therefore, during the last several years, different mutagens have been used by various workers to induce genetic variability in various pulse crops such as *Cicer arietinum* [4-7] *Vicia faba* [8-11] *Vigna mungo* [12-13] *Lens culinaris* [14] *Hordeum vulgare* [15-16] *Vigna unguiculata* [17-18] *Vigna radiata* [19,5] *Glycine max* [20].

As early as 1942, the first disease resistant mutant was reported in barley [21]. This led to the further work on mutagenesis leading to the release of mutants in several crops. Among these varieties, 1468 were of cereals and 370 of legumes. In cereals majority of cultivars came from rice (434), barley (269) and wheat (197) [22]. The induction of mutation has already been recognised as a potential technique for crop improvement since the discovery of mutation effects of X-rays [23-24].

There has been a continuous decline in genetic diversity which eventually has led to induce mutation artificially. In 1927, Muller showed that X-ray irradiation could considerably enhance the mutation rate in *Drosophila*. In 1928, Stadler showed the occurrence of a strong phenotypic variation in barley seedlings and sterility in maize tassels after X-ray exposure in combination with radium. Later on gamma and ionizing radiations which constitute the most commonly used physical mutagens like alpha (α) and beta (β) particles and neutrons were developed at newly established nuclear research centers [25]. During Second World War, radiation-based techniques were used in combination with chemical mutagens that were less destructive, readily available, and easier to work with. In this area, Auerbach and other were Pioneers, who demonstrated an increased mutation frequency in *Drosophila* following exposure to mustard gas [26]. This work was followed by the discovery of chemical mutagens such as sodium azide (SA), methylnitrosourea (MNU) and ethyl methane sulphonate [27]. Chemical mutagens have gained popularity since they are easy to use and can induce mutation at a very high rate [28]. As Compared to radiations, chemical mutagens tend to

induce gene mutations, single-nucleotide polymorphisms (SNPs) rather than chromosomal mutations. Among the chemical mutagens, the most widely used chemical mutagen is EMS (ethyl methane-sulphonate), an alkylating agent. EMS selectively alkylates purines especially guanine causing a thymine base over a cytosine residue opposite to the O-6-ethyl guanine during replication, which results in a point mutation at random [29]. A majority of the alterations in EMS-mutated populations are GC to AT base pair transitions [30] (Table 2; Fig. 4).

The role of mutation breeding in increasing food production and provide sustainable nutrition is well established [31,32] Food security has been variously defined in economic jargon, but the most widely accepted definition is the one by the World Bank “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 [33] 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, the emphasis has been given to physical availability and economic accessibility of food to the people. The mutant varieties have

been grown on large scale 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. In a little less than a century induced mutagenesis is credited with the development of several superior crop varieties that are being grown all over the world (Figs.1-2).

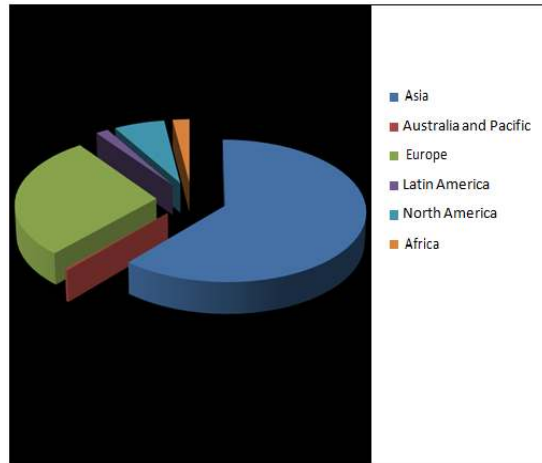


Fig. 1. Cumulative percent of a number of officially released mutants in various regions of the world

Data source: FAO mutant variety database May 2015

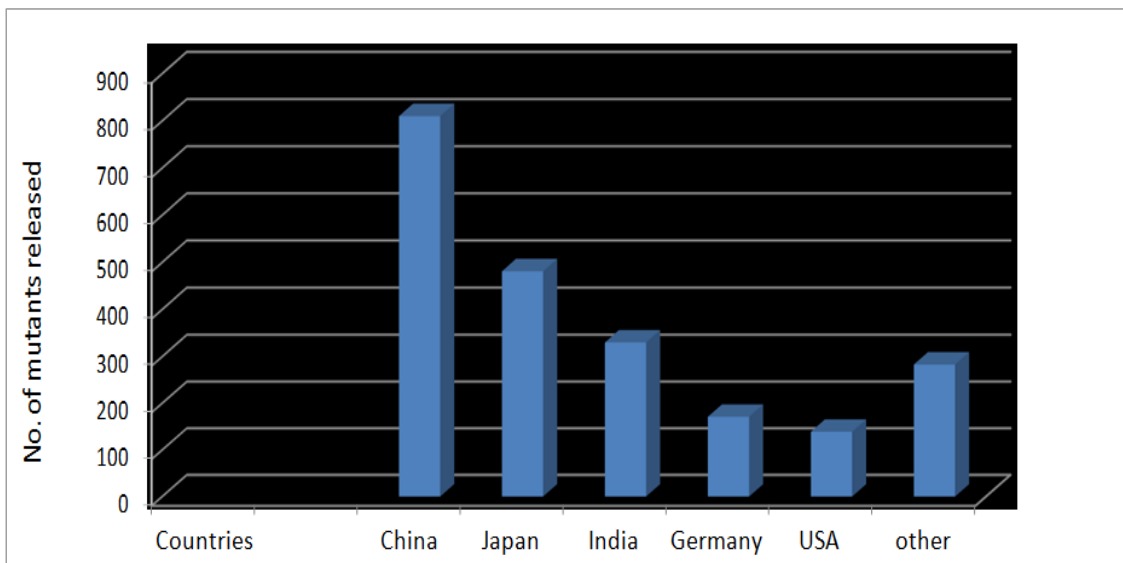


Fig. 2. Total number of released mutant varieties, among top countries

Data source: FAO/IAEA Database of Mutant Variety and Genetic Stocks (<http://mvd.iaea.org>, 2015)

Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques [31]. The use of radioactively labelled probes in recombinant DNA research for cloning and mapping plant genes and transgenesis, particularly for RFLP, microsatellite-based DNA fingerprinting, has become a routine procedure [34]. Recent advances in publically available genomics resources have enabled the use high throughput platform such as TILLING (Targeting Induced Local Lesions in Genomes) in the evaluation of mutant crop varieties for specific sequence genomic alteration. During the last decade, the use of chemically induced mutagenesis has had a renaissance with the development of TILLING technology. In TILLING, mutagenesis is associated with the isolation of chromosomal DNA from every mutant and screening of the population at the molecular level via the advanced molecular techniques [29]. In fact, TILLING uses traditional mutagenesis and nucleotide polymorphism discovery methods for the reverse genetic strategy that is high in throughput, low in cost and applicable to most organisms. Large scale TILLING methods have delivered thousands of induced mutations to the international research community.

Advancements in mutation breeding techniques such as *in vitro* mutagenesis promise to increase further the improvement of crop varieties. Plant breeders have applied *in vitro* culture for rapid multiplication, molecular methods to select desired genotypes, mutagenesis to increase variation, varied environmental conditions to manipulate traits. The use of nuclear techniques in plant breeding has been mostly directed for inducing mutations [35]. Since the discovery of X-rays, the use of ionizing radiation, such as X-rays and gamma rays for creating variation, has become an established technology.

1.1 Past Achievement

In the approximately 80 year-old history of induced mutations, there are many examples of the development of new and valuable alteration in plant characters significantly contributing to increased yield potential of specific crops. The primary motive of the mutation breeding is to enlarge the frequency and spectrum of mutations, [36] and also to

increase the incidence of viable mutations [32]. The main focus has been to upgrade the well-adapted varieties by altering traits like maturity, seed size and disease resistance which play a vital role in increasing yield and yield attributed characters [31]. The attributes that have been improved through mutation breeding include a wide range of characters such as tolerance to abiotic and biotic stresses, duration of maturity and flowering and other yield contributing characters [37]. Cereals and legumes represent the important food crops, improvement in these food crops has been the major concern of plant breeders over the years.

In the past era, these crops have been improved through introduction, selection and hybridisation using either available genetic variability or genetic variability released by recombination. In the present era induced mutagenesis provides an opportunity to create hitherto unknown alleles leading to wide genetic variability. This possibility has been exploited in both cereals and legumes, as is evident from the list of mutant cultivars developed in legumes and cereals (Table 1, Figs. 3-5).

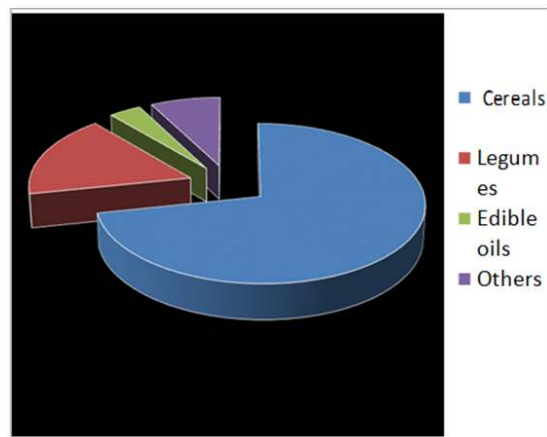


Fig. 3. Number of released varieties developed through mutation breeding in cereals and legumes

Data source: Officially released mutant varieties The FAO-IAEA Database, 2000

1.2 Some Highlight of Mutant Varieties in the World

1.2.1 Genetic enhancement of rice

The impact of induced rice mutants in applied research is best exemplified by the development of improved rice varieties through mutation

breeding. During the past five decades, more than 800 varieties of rice have been developed across the globe, either directly from induced mutations or as a result of crossing such mutants with other breeding lines [38]. The first rice varieties KT 20-74 and SH 30-21, developed through induced mutation, were released in China in 1957 and the first variety Yenhsing-1, developed by a cross-breeding programme with a mutant [39]. Soon afterwards, the semi dwarf mutant Reimei was released in Japan [40] which have significantly increased yield because of their lodging resistance. Calrose 76 and Basmati 370, semi dwarf varieties of rice with short and stiff straw has revolutionised the rice production in USA and Pakistan respectively. In Pakistan, a new variety 'Kashmir Basmati' which matures early and has cold tolerance, and retains the aroma and cooking quality of the parent, was derived from induced mutation in Basmati 370 [41]. Several high yielding rice mutants were released in India under the series PNR and some of these were early in maturity and had short height [42]. Among these, two early ripening and aromatic mutation-derived rice varieties, 'PNR-381' and 'PNR- 102', are popular for cultivation in Haryana and Utter Pradesh. A Rice mutant, 'Zhefu 802' was cultivated on more than 10.6 million ha in China in a span of ten years. In Thailand, gamma ray irradiations expedite the release of an aromatic *indica* variety of rice 'RD6' in 1977. It was extensively grown on 2.4m ha during the year 1994-95. Similar mutant 'RD15', released in 1978, was grown over 0.2 million ha, equivalent to 3.2% of the area under rice ([43] Anonymous, 1995). In Australia nine rice mutant varieties -Amaroo; (1987), 'Bogan' (1987), 'Echua' (1989), 'Harra' (1991), 'Illabong' (1993), 'Jarrah' (1993), 'Langi' (1994), 'Millin' (1995) and 'Namaga' (1997) have been developed. The induction of thermo sensitive genic male-sterile (TGMS) mutant in Japonica rice mutant PL-12, which is controlled by a single recessive gene has an immense contribution in designing the strategies for the production of hybrid rice varieties [44]. In China '26 Zhaizao' was developed by gamma ray irradiation of *indica* rice [45]. These mutants play an important role in two line heterosis breeding.

1.2.2 Developing draught and salinity tolerance in wheat crop

'Sharbati Sonora', a semi dwarf and non-lodging mutant variety has made a significant

contribution to wheat production in India. 'Sharbati Sonora' produced from red grained Mexican variety 'Sonara 60' by gamma irradiation at the Indian Agriculture Research Institute, New Delhi, India. A high yielding mutant Stadler, developed in Missouri, USA had resistance to leaf rust and loose smut, better lodging resistance and early maturity [46]. In Italy Durum wheat cultivation area was significantly expanded due to the cold tolerant mutant varieties.

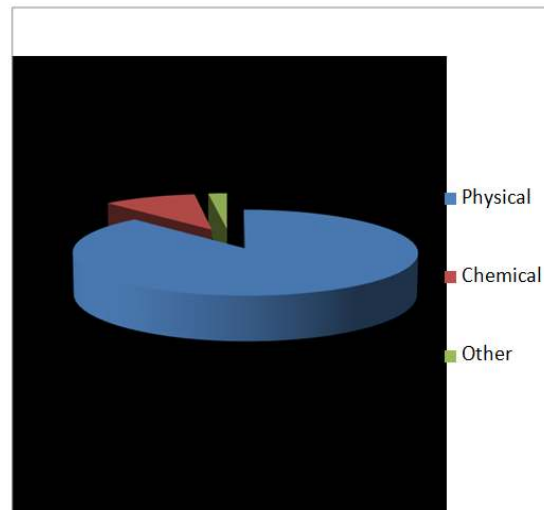


Fig. 4. Comparative use of different mutagens
Data source: Officially released mutant varieties The FAO-IAEA Database, 2015

1.2.3 Enhancing lodging resistance in Barley crop

Mutation breeding has been very successfully used in breeding barley, the introduction of 'Diamant' and 'Golden Promise' a gamma-ray induced semi-dwarf mutant revolutionised brewing industry in Europe. A large number of barley cultivars were developed from crosses involving 'Diamant' in Europe. Since decades these high yielding mutants have been used as the parents of many leading barely varieties released in Europe. Centenario, high yielding, high protein content, early maturity and resistance to yellow rust, was released in 2006 contributes significantly to the food security of the country [47]. 'Luther', gamma ray induced mutant had 20% increased yield, higher tillering and lodging resistance and 'Pennrad', had winter hardiness, better lodging resistance and early ripening [46].

Table 1. Number of officially released mutant varieties in different crop species

Latin name	Number of mutants released
Cereals	
<i>Avena sativa</i> (Oat)	23
<i>Hordium vulgare</i> (Barley)	304
<i>Oryza sativa</i> (Rice)	815
<i>Secale cereal</i> (Rye)	4
<i>Triticum aestivum</i> (Bread wheat)	254
<i>Triticum turgidum</i> (Durum wheat)	31
<i>Zea mays</i> (Maize)	96
Total	1527
Legumes	
<i>Arachis hypogea</i> (Groundnut)	72
<i>Cajanus cajanus</i> (Pigeon pea)	7
<i>Cicer arietinum</i> (Chickpea)	21
<i>Dolichus lablab</i> (Hyacinth bean)	1
<i>Lathyrus sativus</i> (Grass pea)	3
<i>Lens culinaris</i> (Lentil)	13
<i>Glycine max</i> (Soybean)	170
<i>Phaseolus vulgaris</i> (French bean)	59
<i>Pisum sativum</i> (Pea)	34
<i>Trifolium alexadrinum</i> (Egyptian clover)	1
<i>T. incarnatum</i> (Crimson clover)	1
<i>T. pratensis</i> (Red clover)	1
<i>T. subterraneum</i> (Subterranean clover)	1
<i>Vicia faba</i> (Faba bean)	20
<i>Vigna unguicularis</i> (Azuki bean)	3
<i>V. mungo</i> (Black gram)	9
<i>V. radiata</i> (Mungbean)	36
<i>V. unguiculata</i> (Cowpea)	12
Total	462

Data source: FAO/IAEA Mutant variety database, 2015

Table 2. Number of officially released mutant cultivars with different types of mutagens

Mutagen used	Number of released mutant cultivars
Gamma rays	910
X-rays	311
Gamma chronic	61
Fast neutrons	48
Thermal neutrons	22
Ethylmethane sulphonate	106
Sodium azide	11
N-ethyl-N- nitrosourea	57
N- Nitroso-N-methylurea	46

Data source: FAO, 2015

1.2.4 Developing early maturing varieties of peanut

Several peanut mutants (Yueyou No. 5, Yueyou No. 22, Yueyou No. 33, Yueyou 551, Yueyou 187) induced with gamma radiation were released in China as high yielding varieties under the series 'Yueyou', some (Changua No.

4, Lainog, Yueyou 551-38 and Yueyou 551) of those were early in maturity with improved yield. A Mutant peanut variety 'TG 26' developed at Bhabha Atomic Research Centre, Bombay. It is a semi-dwarf plant habit, early maturity, compact pod setting, greater pod bearing, higher harvest index and field tolerance to major diseases [48].

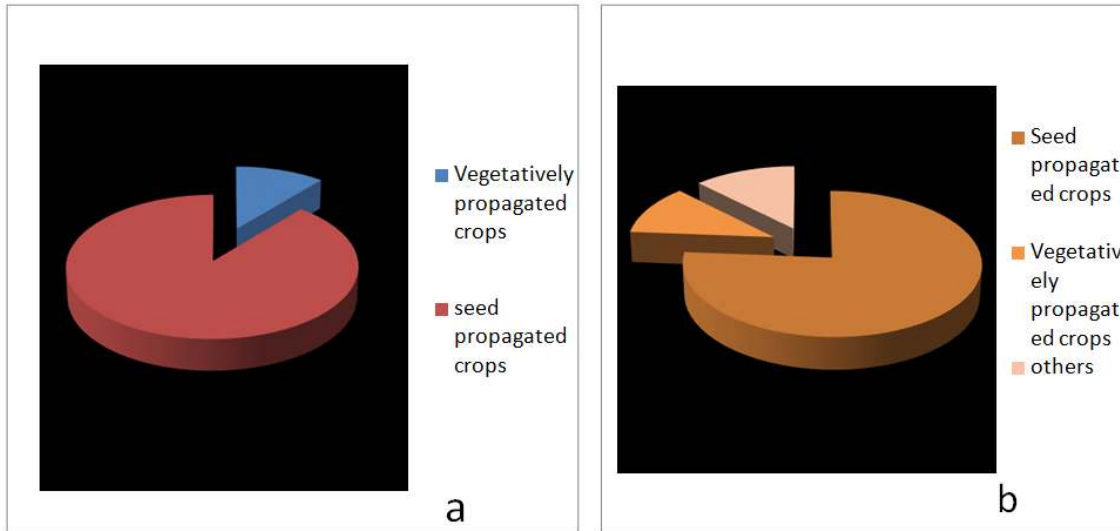


Fig. 5. Officially released mutant varieties by mode of propagation: a, in December 2000; b in May 2015

Data source: *Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations, Rome, 2009, 262-265; FAO/IAEA, 2015*

1.2.5 High yielding and wilt disease resistant chickpea mutants

A series of High Yielding and Wilt Disease Resistant Chickpea Mutants such as Pusa – 408 (Ajay), Pusa – 413 (Atul), Pusa – 417 (Girnar), and Pusa – 547, developed at I.A.R.I., New Delhi, are based on the direct use of induced micro-mutants in a legume crop in the world. Mutant variety Pusa – 547, released in 2006 has thin testa, attractive bold seeds, better cooking quality and high yield performance under late sown conditions of North-Western region of India [49,50].

1.2.6 Ornamental plants

Ornamental plants are ideal for mutation breeding as many valuable characters of economic interest, i.e. growth habitat, flower colour and shape are easily observed after mutagenic treatment. Furthermore, several ornamental plants are heterozygous and propagate vegetatively, hence allowing the detection, selection and conservation of mutants within the M1 generation [51]. The altered traits like flower colour and shape, growth habit and other phenotypes of economic importance were selected [52]. According to the FAO/IAEA mutant variety database, of the 465 mutants developed among the vegetatively propagated plants, most were in the floricultural plants. These included

chrysanthemum (187), *begonia* (25), *Alstroemeria* (35), *dahlia* (34), *Achimenes* (8), *bougainvillea* (9), *rose* (27), *carnation* (18), *azalea* (15) and *Streptocarpus* (30). On the other hand, in fruit trees, very few mutant varieties have been developed. Among these are mutants of apple with altered skin colour in Austria and disease resistant pear in Japan [53,54] seedless grape mutants ‘Rio Red’ and ‘Star Ruby’ in the USA, spineless variety of pineapple was reported in the Philippines [55,56] ‘Novaria’ an early ripening banana mutant with enhanced flavour were developed in Malaysia [57]. Several new varieties of *Chrysanthemum*, *rose*, *carnation*, *bougainvillea* and *Streptocarpus*. Many of these mutants were produced by irradiating culture of apical meristems, auxiliary buds, micro cuttings and embryonic cells and calli suspension. Reports have suggested that the sensitivity to radiation treatment is much more prominent in the case of callus cultures than stem cuttings or seeds.

2. APPLICATIONS IN BASIC RESEARCH

Global food security deteriorated drastically in 1960’s when developing countries like Pakistan and India were desperately short of the food supply. Fortunately, agriculture research responded with a new production technology which has popularly been called as “Green Revolution Technology”. This aided to avoid

large scale starvation for around four decades however, food security problem has again seen a major deterioration in the last few years; sky high food prices and once again poor people of the world are challenged with severe malnutrition the underlining causes that drove to food security deterioration; increasing fertiliser and fuel prices, erratic rain falls, severe drought conditions, excessive floods, divert of food grains into biofuel production will remain for the decades to come. Food security will even get worse since the population is still expanding while no significant increase in arable lands is foreseen. Therefore a newer green revolution is required to solve the problem of food insecurity in the decades to come. The gigantic advent of induced mutation breeding is anticipated to promise a sound solution to further increase food production by both increasing grain production and stability. In this regard, induced mutagenesis is gaining importance in plant molecular biology as a tool to identify and clone genes and to study their structure and function [58]. The application of mutation techniques has generated a vast amount of genetic variability and is playing a significant role in plant breeding and genetics and advanced genomics studies. Recently mutation breeding techniques have also been integrated with other molecular technologies such as molecular marker techniques or high throughput mutation screening techniques are becoming more powerful and effective in breeding crop varieties. Mutation breeding is entering into a new era; molecular mutation breeding. Therefore induced mutation breeding will continue to play a significant role in improving world food security in the coming years and decades. The widespread use of mutation techniques in plant breeding programmes throughout the world has generated thousands of novel crop varieties in hundreds of crop species, and billions of dollars in additional revenue [1] The wide spread use of induced mutations in plant breeding programs has led to the release of elite mutant plant varieties. Such mutants play a significant role in designing crops with improved yield and yield contributing traits, quality and longer shelf life, enhanced stress tolerance and reduced agronomic inputs. The knowledge of biochemistry, physiology and development of plants has rapidly advanced with the introduction of T-DNA insertional mutagenesis. The auxin mutants such as *aux1*, *pid*, *mp* and *lop1* have suggested implications in auxin transport, inhibition, uptake and signal transduction [59]. The understanding mechanism of cytokinin

action was elucidated with the identification of mutants with elevated cytokinin level (*amp1*), photomorphogenic mutant (*det1*, *cop*) cytokinin resistant mutant and cell division mutants [60]. Schmulling et al. in 1997 identified Cytokinin mutants such as *ckr1*, *ein2*, *cry1*, *stp1* and *zea3* in *Arabidopsis thaliana* [61]. These mutants have elucidated the role of cytokinin-regulated genes in diverse biological processes, ranging from cell division, photosynthesis, chloroplast development, disease resistance and nutrient metabolism.

Chandler and Robertson, 1999 elucidated the mechanism of action of growth hormone gibberellin with the screening of dwarf *le* mutant of pea and dwarf mutants of maize [62]. Several dwarf mutants such as *d8* in maize and *Rht3* in wheat are GA deficient and do not respond to applied GA3 [63]. These dwarf mutants have contributed significantly in developing lodging resistant and high fertiliser responsive varieties. Several ABA deficient mutants such as *aba1* in *Arabidopsis* and *aba2* in *N. plumbaginifolia* [64-66] and ethylene response mutants have been isolated [67]. These mutants are highly valuable and have a major role in increasing the shelf life of fruits and extended flower-life and delayed senescence as shown by its transfer to tomato and petunia [68].

A series of homeotic mutants with defective flowers have been identified in *Petunia*, *Antirrhinum* and *Arabidopsis*. The isolation of these mutants has contributed significantly to understand patterns of flower development [69]. Homeotic mutants for leafy cotyledons *lec* are defective in the maturation of embryos which remain green have been developed through insertional mutagenesis [70]. The mutants which determine the development of seed e.g. *fis* mutant have a crucial role in understanding the apomixes [71]. The developmental patterns in crop plants play a vital role in yield and yield attributed traits. The manipulation of these patterns will assume a new dimension in plant breeding in near future.

3. FUTURE PROSPECTS

In recent years interest has rekindled in mutation research since induced mutagenesis is gaining importance in plant molecular biology as a tool to identify and isolate genes and to study their structure and function. These studies will definitely have a major impact on the future crop improvement programmes [72]. Mutation in

association with the new technology of genetic engineering will constitute tools of plant breeders in near future. Although most of the varieties released so far has been developed from a mutation in combination with the direct selection. In the present era *in vitro* culture and molecular methods have resulted in the creation of new and wide paradigm in the utilisation of mutation breeding for crop improvement. Recently, heavy ion beam irradiation has emerged as an effective and efficient way of inducing mutation in many plant varieties because of its broad spectrum and high frequency [73]. In recent years *in vitro* mutagenesis technique has enhanced the crop yield and germplasm innovation by the development of quality and improved resistance traits [74]. In *in vitro* culture techniques, a small amount of tissues and calli can be subjected to mutagenesis for the betterment of crop species [75]. Currently, the use of *in vitro* mutagenesis is low, very little number of plants such as banana and sugarcane have been regenerated through this technique. On the other hand, many seed propagated plants such as wheat, rice, maize and barley can now be regenerated from cell suspension cultures [75]. In future development of *in vitro* cell selection techniques for disease resistance would be equally important. A coordination of the recent techniques of anther and microspore culture, cell suspension, irradiation of haploid cells and chromosome doubling and regeneration of doubled haploid plants could be utilized to obtain genotypes with desired traits [76].

The induced mutation has also proved useful in the preparation of genetic maps that will facilitate molecular marker assisted plant breeding in future [77]. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement [78]. The direct use of mutation in the development of molecular maps in structural and functional genomics could lead to rapid improvement of plant yield and quality. The molecular techniques of DNA fingerprinting and molecular mappings such as RAPD (Random Amplified Polymorphic DNA,) AFLP (Amplified Fragment Length Polymorphisms) and STMS (Sequence-Tagged Microsatellite Sites) have contributed significantly in the screening and analysis of mutants. Site directed insertion of transgenes based on chimeric RNA/DNA oligonucleotides as done in tomato [79] and maize and mutant tagging will be widely used in gene technology [80].

4. CONCLUSION

At present genetic variability is narrowed using conventional breeding approaches for a long period, induced mutagenesis are one of the most important approaches for broadening the genetic variation and diversity in crops to circumvent the bottleneck conditions. Induced mutagenesis, albeit almost a seven decades old technique, demonstrably can contribute to unleashing the potentials of plant genetic resources and thereby avail plant breeders the raw materials required to generate the envisaged smart crop varieties. Crop varieties generated through the exploitations of mutation breeding are significantly contributing to global food and nutritional security and improved livelihoods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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