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Role of Induced Mutations for Enhancing Nutrition Quality and Production of Food

Nihar Ranjan Chakraborty* **and Amitava Paul**

Department of Crop Improvement Horticulture and Agricultural Botany, Palli Siksha Bhavana, Visva-Bharati, Sriniketan, West Bengal (731 236), India

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Correspondence to

*E-mail: nrchakraborty@gmail.com

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Abstract

Crop plants form the major components of human diets, providing the required calories and nutrients to sustain life. World is facing a food and energy crisis of unprecedented proportions. Food production, to meet the needs of the growing world population, can be augmented by improving agronomic techniques and by growing genetically improved cultivars of a wide range of crops. Induced mutations are a proven tool in creating a wealth of desirable genetic variability in plants and can be a catalyst in developing improved crop varieties with nutrition quality, and higher yield. The commercial utilization of approximately 3,000 mutants strongly shows that mutation breeding is a useful tool for generating new germplasm for crop improvement. Induced mutations will continue to have an increasing role in creating crop varieties with traits such as modified oil, protein and starch quality, to the enhancement of phytonutrients in fruits and reduction of anti-nutrients in staple foods.

1. Introduction

The world food problems are very serious matter now-a-days. The FAO estimates that about 800 m people do not have enough food to eat, while the world population in increasing by around 80 m each year, with most of this increase in least developing countries. The world population is expected to reach 7 b in the next 20 years and 10 b by 2050. Population bloom of the world has triggered the necessity to increase production level for feeding the extra mouth. Global food security continues to be the centre stage issue and plant breeders are under pressure to sustain the food production to meet the demand of ever growing human population. Several factors such as abiotic and biotic stresses, industrial pollution, deforestation, loss of genetic diversity, water shortages and so on are responsible for having a negative impact on food production. Malnutrition and hunger continue to be some of the most serious problems facing society. Malnutrition with respect to vitamin A and micronutrients like, iron, and zinc affects more than 40% of the world's population. Micronutrient deficiencies are common in many developing countries and are typically due to inadequate food intake, poor dietary quality and poor bioavailability. For example in wheat, zinc is quite low in grain and consequently deficient in human diet among the developing countries, and to supplement it needs zinc enriched wheat grains at the farmer's field (Hussain et al., 2010). World major crops like rice, wheat, barley, etc. would require continuous modifications for sustainable food production, where as nutritional security would be maintained by improving legumes, vegetables, and fruits.

Induced mutations have had a large impact in transforming the agriculture of the world, particularly in generating crop species having desirable traits (Ahloowalia et al., 2004). Examples of improved traits with high impact are those which alter the architecture of the plant. Architectural changes include alteration in branching pattern and reduction in plant height. The major achievement of the Green Revolution in 1960s and 1970s was due to the introduction of semi-dwarf cultivars of wheat and rice along with crop production packages such as controlled use of irrigation, fertilizer, herbicide and fungicide. Semi-dwarf varieties have been produced by exploiting natural nucleotide variation and through induced mutations, and have led to tremendous increases in productivity.

Plant breeders and geneticists are making all efforts to sustain food production and nutrition quality of food to make genetic improvement of plants by using conventional and modern tools. The genetic variability is highly desirable for developing new cultivars, which is induced by mutagen treatments and natural spontaneous change. The spontaneous mutation rate is pretty low and can not be exploited for breeding and that is why artificially mutations are induced with physical and chemical mutagen treatment. Many useful genetic changes have been induced by mutagen treatment including high yield, flower color, disease resistance, and early maturation and so on in crop, vegetables, medicinal herbs, fruit and ornamental plants. So far, over 3,000 mutant varieties have been officially released from 170 different plant species in more than 60 countries throughout the world including rice, wheat, barley, sorghum, legumes, cotton, edible oil, ornamental plants and fruits (www-mvd.iaea.org). In vegetatively propagated crops, where genetic variation is limited, mutation induction is a tool of choice to be promoted. Mutation induction allows for overcoming the deadlock of sterility and parthenocarpy by creating useful variants. In fruit crops, mutagenesis has been quite useful in isolation of useful mutants such as plant size, blooming time, fruit ripening, fruit colour, and resistance to pathogens. Another major fruit crop is date palm (*Phoenix dactylifera* L.), a major source of human nutrition including vitamins, sugars, fat, salts and minerals, and oils; and has high potential to produce bio-ethanol (Jain, 2011). China and India are the major producers of mutant varieties to feed their ever growing human population. Among all crops, the released highest number of mutant varieties is reported in rice.

2. Strategy for Production of New Mutant Variety

The process of utilization of induced mutations is broken up into four main parts: inducing mutations at a desired density and spectrum, dissolving chimeric sectors, phenotyping, and genotyping. Each of these 'modules' can be adapted to fit a specific crop or propagation strategy (seed or vegetative) and integrated into a pipeline to enhance the efficiency of mutation assisted breeding. The prime strategy in mutation 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 increase grain yield because of their resistance to lodging and high planting density, although it involves high doses of nitrogen application. The maturity of some mutants resulted in timely planting of the follow-up crop; for example early maturity of cotton allowed early planting of the wheat crop, resulting in higher wheat yield. The induction of thermo-sensitive genic male-sterility 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.

3. How Mutation Breeding is Beneficial?

Mutation breeding is particularly advantageous where conventional hybridization method is difficult or not useful. One of the most important aspects of mutation breeding have been the quick rectification of defects in varieties and advanced breeding lines, induction of polygenic mutations and development of ideotypes for various agro-climatic conditions. It has been demonstrated that induced mutation can increase yield as well as other agronomic characters such as stiffness of straw, time of maturity, adaptability, shattering resistance, disease resistance, protein content, baking quality, malting quality and numerous other characters (Borojevic, 1990; Brunner, 1991). Mutation breeding generally targets those traits that have either not been favored by natural selection in the evolutionary process or have not been improved during previous plant breeding efforts. Sigurbjornsson and Micke (1974) have shown the increasing role of induced mutation in crop improvement.

One of the greatest drawbacks of mutation breeding, however, is the undesirable effect produced by the pleiotropic action of the mutant gene or simultaneous mutation of closely linked genes. It is a fact that most of the induced mutations are deleterious, but when appropriate selection technique is applied, useful mutants can be recovered. Nevertheless, the occurrence of even a few desirable mutation in high yielding varieties has the great advantage of becoming homozygous and expressing its superiority within a couple of generations after induction in M_2 or M_3 as compared to F_6 or F_7 generations in case of hybridization.

Thus, induced mutations can provide useful alternative or complement to natural variation as well as to hybridization. Induced mutations may be similar to those, which occurred naturally or many of which, probably, have never occurred spontaneously or have been lost from the natural population. By applying appropriate selection techniques, desirable mutants suitable for modern agricultural system could be retained (Brock, 1971).

4. Crop Improvement through Induced Mutations

Crop improvement programs through induced mutations were about eight decades ago, immediately after the discovery of mutagenic actions of X-rays on *Drosophila* by Muller (1927) and in barley by Stadler (1928), initiated a new field of induced mutagenesis (Ahloowalia et al., 2004). A major breakthrough in the mutation breeding was achieved by the classic work by Gustafsson (1940), in which large number of mutations in barley, especially for chlorophyll mutations and stiffness of straw were reported. Today, the plant breeders have at hand, a number of effective physical and chemical mutagens which are capable of inducing variation when applied properly. The spectrum and frequency of mutation depends on the choice of mutagens and their dosage used. Chemical mutagens produce a higher rate of gene mutation but their penetration to the relevant target is quite uncertain. Besides, poor reproducibility, persistence of mutagens or its metabolite in treated material and the risks of safe handling are the matters of great concern. The physical mutagens, such as X-rays and gamma rays are widely used because of their high penetration and precision. Among the various mutagenic agents used for developing varieties, a great majority (1,411 out of 1,585) of directly developed mutant varieties were obtained with the use of radiations, particularly gamma rays (910 mutant varieties) as mutagens (Kharkwal et al., 2004). Induced mutation, thus, have played a pivotal role in enhancing world food security, since new food crop varieties with various induced mutations have contributed to the significant increase of crop production (Kharkwal and Shu, 2010).

In India, sustained efforts for crop improvement through induced mutation were initiated during the second half of the 1950s, although the world's very first mutant variety of cotton, MA-9 induced by X-rays, endued with drought tolerance, was released in 1948 in India (Kharkwal et al., 2004). Mutation breeding is being carried out in several national/ state universities/institutes like Indian Agricultural Research Institute (IARI), New Delhi; Bhabha Atomic Research Center (BARC), Mumbai; ICRISAT, Hyderabad; Tamil Nadu Agricultural University (TNAU), Coimbatore; and National Botanical Research Institute (NBRI), Lucknow. According to Kharkwal and Shu (2010) more than ₹258 crores of income was generated by India due to release of 343 mutant cultivars belonging to 57 plant species. Detailed information regarding number of mutant varieties released for cultivation in India and total number of varieties released in each crop is presented in Table 1. Some popular mutant varieties which are cultivated in India are mentioned in Table 2.

5. Induced Mutation for Nutrition and Quality Improvement

Induced mutations have played a great role in increasing quality and nutrition components of a crop. There is a necessity to enhance mineral elements and amino acids essential for human and animals, alteration of protein and fatty acids profiles for nutritional and health purposes. Of the 3,000 mutant varieties developed globally, 776 mutants have been induced for nutritional quality (www-mvd.iaea.org). Several mutant genes have been successfully introduced into commercial crop varieties that significantly enhance the nutritional value of those crops. Collaborative research programme under Food & Agriculture Organization and International Atomic Energy Agency (FAO/ IAEA) has been focussed on at crop improvement by induced mutation using nuclear techniques (Jain, 2000) intended to produce strains of cereals with higher concentrations of micronutrients and improvement of their bioavailability by reduction in the concentration of phytic acid. In this regard, strategies should be aimed at breeding plants that can contain high levels of minerals and vitamins in their edible parts to reduce substantially the recurrent costs associated with fortification and supplementation (Shetty, 2009). Such a strategy will be

Table 1: Mutant varieties of different crops released for cultivation in India

Crop	No. of varieties	Specific crop and no. of varieties
Cereals	74	Rice (42), barley (13), pearl millet (5), finger millet (7), foxtail millet(1), wheat (4), sorghum (2)
pulses	57	Mungbean (15), blackgram (9), chickpea (8), cowpea (10), mothbean (5), pea (1), pigeonpea (5), frenchbean (1), lentil (3)
Oilseeds	44	Groundnut (18), mustard (9), castor bean (4), sesame (5), soybean (7), sunflower (1)
Fibre crops	14	American cotton (8), tossa jute (3), white jute (2), dseicotton (1)
Vegetables	14	Tomato (4), turmeric (2), bitter gourd (1), brinjal (1), green pepper (1), okra (2), ridge gourd (1), snake gourd (1), cluster bean (1)
Cash crops	10	Sugarcane (9), tobacco (1)
Medical crops	17	Citronella (9), German chamomile (1), Indian henbane (1), isabgol (2), khasianum (1), opium poppy (2), spearmint (1)
Fruit trees	2	Mulberry (1), papaya (1)
Forage crops	1	Egyptian clover (1)
Ornamentals	110	Chrysanthemum (49), rose(16), dahlia (11), portulaca (11), bougainvillea (13), wild sage (3), gladiolus(2), *Hibiscus* sp. (2), tuberose (2), coleus (1)
Total	343	

Table 2: Some popular mutant variety cultivated in India

Common name	Latin name	Mutant cultivar
Rice	*Oryza sativa*	PNR-381, PNR-102
Wheat	*Triticum aestivum*	NP-836, Sharboti Sonora
Sugarcane	*Sachharum officinarum*	Co-6608
Castor	*Ricinus communis*	Aruna
Blackgrame	*Vigna mungo*	TAU 1,TU 94-2
Cowpea	*Vigna unguiculata*	V-16 (Amba)
Mungbean	*Vigna radiate*	TAP-7, MUM-2, BM-4, LGG-409, LGG-450, Co 4, Dhauli, Pant moong-1, TM 96-2
Chickpea	*Cicer arietinum*	Pusa-547, Pusa-408 (Ajoy), Pusa-413 (Atul), Pusa-417 (Girnar)
Jute	*Corchorus capsularis*	Shymali

successful depending on farmer's willingness to adopt such varieties, palatability of the edible parts of these varieties and consumer acceptability, and if the incorporated micronutrients can be absorbed by the human body (Bouis, 2002). Certain considerations need to be addressed before a plant breeding strategy can be put in place to combat micronutrient deficiency to function and to have universally adoptability, particularly in Developing Countries (Bouis, 2002). These include, feasibility to breed micronutrient- dense staple food varieties, effects on plant yields and farmer's adoption of such varieties, possibility of changes that micronutrient density can have a great nutritional balance on the staple diet of consumers, bioavailability of extra micronutrients in staple foods to humans, and alternate options for more easily sustainable strategies for reducing micronutrient malnutrition. A few examples are given below:

5.1. *Quality protein maize*

Maize endosperm protein is deficient in two essential amino acids, lysine and tryptophan. The opaque two mutant genes, together with endosperm and amino acid modifier genes, was used for the development of QPM varieties. QPM has almost twice as much lysine and tryptophan, and 30% less leucine, as normal maize, and has shown to have dramatic effects on human and animal nutrition, growth and performance. QPM varieties are now grown on hundreds of hectare land.

5.2. *Low phytic acid (LPA) crops*

Much of the phosphorus is deposited as phytic acid and its salt form (phytate) in seeds. Since phosphorus and mineral elements such as iron and zinc in the form of phytate cannot be digested by humans and monogastric animals, reduction of phytic acid would increase the bioavailability of phosphorus and micronutrient mineral elements. New mutants varieties of barley, wheat, rice and soybean with low phytic acid (LPA) have been released and has facilitated to reduce both phosphorous pollution and increase bioavailability of phosphorous and micronutrient minerals in cereals and legumes.

5.3. *Oilseeds with optimized fatty acid compositions*

The optimal composition of plant oils depends on their end uses, for example, unsaturated fatty acids (oleic, linolenic) are desirable for salad and cooking oils, but increased concentration of saturated fatty acids (stearic, palmitic) is preferred for oils used in the food industry, since high temperature processes (frying) require oils resistant to thermo-oxidation. By using the mutated genes, new varieties have been developed for numerous purposes.

6. **Relevance of Biotechnology and Biofortification**

Three major micronutrient deficiencies that have been identified in humans include vitamin A deficiency, iron deficiency and iodine deficiency (Prasad, 2010). Micronutrient deficiency of Zn has also received global attention (Hussain et al, 2010). In addition, improving the amount of essential amino acids in important staple foods, such as rice, has gained interest (Welch and Graham, 2004). Rice is also one of the priority crops for enhancement of the nutritional factors such as vitamin A, Zn and iron through international schemes such as Harvest Plus (Pfeiffer and McClafferty, 2007). Increase in bioavailability of Fe in rice has been investigated by transferring a gene for heat resistant phytase from fungal sources that degrades phytate in plant (Bhat and Vasanthi, 2005) which may also increase the Zn bioavailability in rice.

An adequate concentration of micronutrients and their availability seem to be essentially required in major staple crops if these crops are considered to provide a sustainable solution to the problem of malnutrition (Pinstrup-Anderson and Pandya-Lorch, 2001). This holds true for cereals since majority of the population in the developing world depend on cereal based food intake. Rice alone contributes to 23% of the energy consumed worldwide and countries that rely heavily on rice as the main staple food often consume up to 60% of their daily energy from this cereal (Khush, 2003). Conventionally, nutrient content of crops can be improved by using field fortification strategies, to enhance the micronutrient and trace element content of crops by applying enriched fertilizers to the soil. Biotechnological tools have generated new opportunities to improve the amount and availability of nutrients in plant crops. These include simple plant selection for varieties with high nutrient concentration in the seeds, cross-breeding for incorporating a desired trait within a plant, and genetic engineering to manipulate the nutrient content of the plant (King 2002). One of the successful examples in using the genetic engineering approach is the production of "Golden Rice" involving the transfer of the genes necessary for the accumulation of Carotenoids (vitamin A precursors) in the endosperm that are not available in the rice gene pool. The first generation Golden Rice with a gene from daffodil and a common soil bacterium drew considerable criticism as a technological solution to a problem associated with poverty and hunger. It was argued that Golden Rice would encourage people to rely on a single food rather than the promotion of dietary diversification. The development of Golden Rice 2 by replacing the daffodil gene with an equivalent gene from maize increased the amount of beta carotene by about 20-fold resulting in about 140 grams of the rice providing a child's RDA for beta carotene (Raney and Pingali, 2007). It has also been demonstrated that beta carotene from golden rice is efficiently converted to vitamin A in humans (Tang et al., 2009).

7. New Techniques for Mutation Induction, Screening and Utilization

There have been substantial technological developments in the induction, screening, and utilization of mutated genes. Traditionally, plant materials are treated with physical or chemical mutagens, such as Gamma-rays, ethyl methane sulphonate (EMS), and mutants are screened from the progenies in the field for morphological traits, or in the laboratory for chemical components. Due to the usually low mutation frequency, large populations need to be screened, which makes it rather expensive and sometimes impossible to identify a target mutation. The new developments for mutation induction include the use of space flight, ion irradiation, and transposable elements for mutation induction, the use of restriction endonuclease for site directed, homologous recombination; DNA markers linked to mutated genes for marker-assisted selection and tracing of the gene, and TILLING (Target Induced Local Lesions IN Genome), as well as different variant versions for high throughput screening of mutated alleles. TILLING is a high-throughput and low cost reverse genetic method for the discovery of induced mutations. The method has been modified for the identification of natural nucleotide polymorphisms, a process called Ecotilling. The methods are general and have been applied to many species, including a variety of different crops.

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 developments 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.

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.

A range of several mutants in different ornamental plants, maize, rice and wheat have been isolated and used for crop breeding. Similarly low energy ion beam (LIB) has been used for mutation breeding and gene transfer. This method has many advantages such as low damage rate, higher mutation rate and wider mutation spectrum. In rice, 11 new lines of rice mutants with higher yield, broader disease resistance, and shorter growing period and high grain quality were developed and now being cultivated in China. In jasmine rice from Thailand, a wide range of mutants were recorded including short stature, red/purple colour of leaf sheath, collar, auricles, ligules and dark brown stripes on leaf blade, dark brown seed coat and pericarp (Mohan jain and Suprasanna, 2011).

8. Economic Impact of New Mutant Variety

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. The economic value of a new variety can be assessed from several parameters. These include - increased yield, enhanced quality, reduced use of pesticides and fungicides (resistant varieties to diseases and insect pest), saving in water (short duration crop, drought tolerance), increased nutritive value, high lysine and vitamins, increased oil-shelf life, reduced toxins, etc.

It is clearly established that the plant cultivars derived from induced mutations have contributed billions of dollars to the economies of many countries. The beneficiaries have been not only developing countries (India, China and Pakistan), but also North American and European countries have gained from the release of mutant cultivars (Ahloowalia et al., 2004). 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.

9. Conclusion

Induced mutation has come to stay as an efficient plant breeding method towards improvement of crops and development of commercial varieties in achieving the target of food self-sufficiency and nutritional security. The diet of many urban and rural poor is deficient in several nutrients, vitamins and minerals. Diversification to nutrient-rich millets, fruit and vegetables product can go a long way in bridging the gap in nutrient intakes provided that these foods become accessible to the poor.

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