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Induced mutation- a tool for creation of genetic variability in rice (*Oryza sativa* L.)

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ABSTRACT

The genetic variability in plants is getting narrowed due to human activities and natural calamities. Mutation is defined as sudden heritable change in a characteristic of an organism. Induced mutation is one of the potential tools for generating more variability which has been successfully exploited for improving several traits in many crop plants including rice (Oryza sativa L.). Rice is the world's leading food grain crop which serves as the main source of calories consumed by human being and occupies almost one-fifth of the total land area covered under cereals. The demand of rice is continuously increasing due to ever-growing population. To meet the growing demand, more variability is required to develop new high yielding varieties with tolerance to biotic and abiotic stresses. Use of induced mutation for improvement of rice received importance as a potential tool in the late sixties. A wide array of physical and chemical mutagens have been evaluated on rice and based on their relative efficiency and effectiveness, x-rays, γ- rays and fast neutrons(among physical) and EMS, nitroso methyl urea (NMU), sodium azide and ethidium bromide (among chemical) have been found useful for obtaining desirable mutants. Now, combination of both, chemical (EMS) and physical (γ- rays) mutagens are also being used which are giving encouraging results in creating variability and developing high yielding varieties. This method is generally being used for improvement of one or two simply inherited traits in otherwise well adapted and highly productive genotypes. The traits which have been induced in rice through mutation in the last five decades are; early maturity, short stature, more number of tillers, resistance to blast and leaf blight, resistance to lodging and cold, improved grain quality and higher yield. The present article gives an insight into the relevance of induced mutation in creation of genetic variability and development of outstanding rice varieties.

Keywords: Mutagens, mutation, rice

Increasing crop yields to ensure food security is a major challenge. Amongst the obstacles against this, are the changing climate (increasing temperatures and more erratic rainfall) which most often compromise crop productivity (Parry *et al.*, 2005) and the need to produce additional food and crops for bioenergy while minimizing the carbon costs of production (Powlson *et al.*, 2005). There is, therefore an urgent requirement for new higher yielding varieties (Parry *et al.*, 2007; Reynolds *et al.*, 2009) with improved nutrient (Lea and Azevedo, 2006) and water use efficiency (Richards, 2000). Improved varieties or hybrids of cereals and millets helped in ushering the green revolution. The ultimate goal of a plant breeder is to increase the yield potential of a crop, coupled with desirable quality of grain and this is only possible through the extent of genetic variability available in the plant population largely which determines the efficiency of selection. Thus, insight into the magnitude of genetic variability is of paramount importance to the plant breeder for starting a judicious breeding programme. Exploiting natural or induced genetic variability is a proven strategy in the improvement of all major food crops, and the use of mutagens to create novel variation is particularly valuable in the crops with restricted genetic variability and also at places where long breeding procedures is not desired and aim is to develop a good plant type in a shorter duration of time.

Early history of induced mutations

Variations in the individuals are either induced by the environment through hybridization, mutation and polyploidization or by artificial means using the similar principles. The variations or changes which suddenly appear in an individual and can be inherited to the progeny, is called as mutation. According to Hugo De Vries (1886) 'mutation' is defined as any sudden and drastic heritable change in gene which is not traceable or ascribable to segregation or recombination', but, mutations were known to occur in animals and plants much before this time. For example, a short-legged sheep was discovered by an English farmer in the 18th century; this sheep was used to establish a breed named Ancon. With the discovery of X-rays, induced mutations in Drosophila by Muller in 1927, and in barley by Stadler in 1928, a new era in the field of genetic research was opened.

Spontaneous and induced mutations

Mutations may be induced or natural which brings changes in the genetic makeup of an organism either at chromosome level (chromosomal mutations) or at gene level (gene or point mutations). It may be structural, compositional or numerical changes which cause the change in character of an individual.

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Mutation plays important role in plant breeding and also displays the key role in evolution of species. Spontaneously occurring mutations have been of great value to plant breeders and have been used in the development of superior varieties of cereals, vegetables, forage crops, fruit trees and ornamental plants. The limitation on making much greater use and selection of them is the extreme rarity of their occurrence and because of the several repair mechanisms that organisms possess. On the other hand when one applies ionizing radiations or chemical agents, it is possible to increase the rate of mutation so that every plant in a treated population will contain at least one mutation. One of the important advantages is that induced mutations enable breeders to introduce specific improvements without affecting the existing good attributes of crop varieties. It is not surprising that the enormous potential for usage of induced mutations in plant breeding has stirred the imaginations of geneticists from the turn of the19th century. Through better understanding of the process the potential of induced mutations is now being realized. Induction of mutations, primarily a method of generating variation, can contribute to plant improvement when combined with selection, or recombination and selection, or with other methods of manipulating genetic variation.

Mutations in crop breeding

Plant breeding not only aims at improving the crop productivity but also improves the quality characters through changes in heredity. The utilization of artificially induced mutations, through mutagenic agents, for the development of superior varieties in plants is known as mutation breeding. The history of mutation breeding in India started in 1935 at Bose Research Institute, Kolkata and later established at IARI, New Delhi in 1959. Mutation breeding has been used in a number of cereal, pulse, oilseed, vegetable and ornamental crops, but has played an important role in development of varieties with varied traits in cereals, particularly in rice.

In India, sustained efforts for crop improvement through induced mutations were initiated during the second half of the 1950s, although the world’s very first mutant variety of cotton, MA-9 induced by X-rays, endued with drought tolerance, was released in 1948 (Kharkwal *et al.,* 2004). The Indian Agricultural Research Institute (IARI) , New Delhi; Bhabha Atomic Research Center (BARC), Trombay, Mumbai; Tamil Nadu Agricultural University (TNAU), Coimbatore and the National Botanical Research Institute (NBRI), Lucknow, are some of the major research centers actively involved in mutation breeding for several crops and have contributed substantially to the development and release of a large number of mutant varieties.

Choice of mutagens

Agents that induce mutations are known as mutagens. Mutagens are different kinds of radiations (physical mutagens) or certain chemicals (chemical mutagens). The physical mutagens comprises of ionizing radiation *viz.*, particulate (α rays, β rays, fast neutrons and thermal neutrons) and non-particulate also called as electromagnetic radiation (X rays and γ rays). The non-ionizing comprises of UV radiation. The other class is of the chemical mutagens consisting of alkylating agents (sulphur mustards, nitrogen mustards, epoxides, imines, ethyl methane sulphonate or abbreviated as EMS etc.), acridine dyes (acriflavine, proflavine, acridine orange, acridine yellow, ethidium bromide), base analogues (5- bromouracil, 5-chlorouracil) and others which includes nitrous acid, hydroxyl amine and sodium azide. Out of these, the most commonly used mutagens are the gamma rays, ethyl methane sulphonate (EMS) and sodium azide. Mutation induction with radiation is the most frequently used method to develop direct mutant varieties (Ahloowalia and Maluszynski, 2001). The usefulness of mutagens in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagen), but also on its mutagenic efficiency in relation to undesirable changes like sterility, lethality, injury etc (Girija and Dhanavel, 2009). The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations (Smith, 1972; Awan and Bari, 1979; Reddy and Rao, 1988; Bansal *et al.,* 1990; Katoch *et al.,* 1992; Pillai *et al.,* 1993; Solanki *et al.,* 1994; Kumar, 1998). With the advancement in science, different mutagens were discovered capable of inducing mutations, as described above. Different mutagens cause mutations in crops but, in specific ways (Gustaffson, 1940).

Mode of action of physical mutagens

Radiation was the first known mutagenic agent; its effects on genes were first reported in 1920's. Roentgen discovered X-rays in 1895, Becquerel discovered radioactivity in 1896, and Marie and Pierre Curie discovered radioactive elements in 1898. These three discoveries and others led to the birth of atomic physics and our understanding of electromagnetic radiation.

The ionizing physical mutagens react by production of highly reactive free radicals; interaction takes place between the radicals and DNA, proteins and lipids in cell membrane thus causing alterations. When interacting with DNA, causes deletions or chromosome loss, rearrangements and loss of bases thus altering all together the structure and function of proteins and consequently changes in phenotype. The non-ionizing mutagen *viz.,* UV rays leads to the formation of thymine dimers, these dimers cause the strand to buckle, disrupting normal base pairing and thus prevent normal replication and transcription.

Mode of action of chemical mutagens

The first report of mutagenic action of a chemical was in 1942 by Charlotte Auerbach, who showed that nitrogen mustard (component of poisonous mustard gas used in World Wars I and II) could cause mutations in cells. The alkylating agents' causes reaction with bases and add methyl or ethyl groups. Depending on the affected atom, the alkylated base may then degrade to yield a baseless site, which is mutagenic and recombinogenic, or mispair to result in mutations upon DNA replication. The acridine dyes are flat, multiple ring molecules which interact with bases of DNA and insert between them. This insertion causes a "stretching" of the DNA duplex and the DNA polymerase is "fooled" into inserting an extra base opposite an intercalated molecule resulting in frameshifts. Base analogues structurally resemble purines and pyrimidines and may be incorporated into DNA in place of the normal bases during DNA replication.

Mutations for improvement in rice

Rice [*Oryza sativa* (L): Family- Poaceae (2n = 24)] is the most important staple food crop of the world and serves as the main source of calories consumed by human and occupies almost one-fifth of the total land area covered under cereals. The genus *Oryza* probably originated in the humid regions of the Gondwanaland supercontinents (Chang, 1976). It was originally cultivated in tropical Asia, the oldest record dating 5000 years BC, but then extended also to temperate regions (Watanabe, 1997). Genus *Oryza* contains approximately 22 species of which 20 are wild species and 2 (*Oryza sativa and Oryza glaberrima*) are cultivated and grown worldwide including in Asian, North and South America, European union, Middle Eastern and African countries. Cultivated varieties of *Oryza sativa* are evolved into 3 ecotypes; *indica* (tropics and sub tropics), *japonica* (grown in Japan, Korea and Northern China) and *javanica* (grown in Indonesia). Current cultivation for *Oryza sativa* is worldwide extending from latitude 35^0 S to 50^0N (Northern China) over 110 countries, 90% of all rice grown and consumed in Asia. In India, it is cultivated in an area of 44.10 million hectares with a production of 104.32 million tonnes and productivity of 3.45 tonnes ha^{-1} (Source: Foreign Ag. Service, USDA, Office of Global analysis, April, 2012).

Rice production and productivity was significantly enhanced with the introduction and cultivation of semi-dwarf, fertilizer responsive, non-lodging and high yielding varieties in the early seventies leading to the "Green Revolution". The yield level of high yielding varieties is plateauing in recent years. To meet the demand of increasing population and sustain self sufficiency, rice production needs to be increased up to 130 million tonnes by 2030. That can be achieved only by increasing the rice production by over 2 million tonnes per year in coming decade (FAO, 2012). This has to be achieved against the backdrop of declining the natural resources like land, water and other inputs and without adversely affecting the quality of environment.

There is an urgent need to adopt some innovative technologies to break the yield ceiling in rice or to assist conventional breeding programmes to develop good varieties. Historically the use of mutagenesis in breeding has involved forward genetic screens and the selection of individual mutants with improved traits and their incorporation into breeding programmes. Inducible mutation is a suitable source of producing variation for crop improvement (Domingo *et al.*, 2007). Several improved mutant varieties with high economic value have been released via mutation breeding programme (Din *et al.*, 2004). One of the reasons for the low yield is that the most cultivated rice are traditional cultivars that are tall, with long maturation period, prone to lodging and moreover susceptible to pests and diseases. In order to address the above constraints, mutation breeding has been used to upgrade the well-adapted local varieties by altering one or two major traits, which limit their productivity or enhance their quality value.

Semi-dwarfism and earliness are the characters most frequently desired in released rice mutant cultivars, although other traits such as higher stem number, improved grain quality, blast tolerance, photoperiod insensitivity and salt tolerance are also common (Maluszynski and Ahloowalia, 1995). Germination and survival is linearly decreasing with increase of radiation dose in case of γ- rays and fast neutrons (Din *et al.*, 2004). In rice, to allow 60% survival of seeds (percentage of seeds germinated and developed to adult plants) and effective dosage of gamma rays generally ranged from 150 to 300 Gy (Rutger, 1992). The relationship between the two units of radiation *viz.*, Gray and kilo Roentgen is 10 Gy = 1 kR.

Table 1: Mutant rice varieties released in India for cultivation5

Sl. No.	Name of variety	Parentage	Year of release	Duration (days)	Recommended for Cultivation
1.	Jagannath / (BSS-873)	Mutant of T-141	1975-76	140-160	Coastal region of Andhra Pradesh.
2.	CNM-25	Mutant of IR-8	1978	103 -148	West Bengal.
3.	CNM-31	Mutant of IR-8	1978	135 -155	West Bengal.
4.	Lakshmi / (CNM-6)	Mutant of IR-8	1982	105-110	West Bengal.
5.	Biraj / (CNM-539)	Mutant	1984	165-170	West Bengal.
6.	Prabhavati /(PBN-1)	Mutant of Ambemohar local variety	1985	115-120	Maharashtra
7.	Padmini /(IET-10561)	Mutant selection from the CR-1014 variety	1989	140	All India
8.	Lunisree / (IET-10678)	Mutant selection from Nonasail variety	1992	145	Orissa
9.	Pusa NR-381 /(IET-9208)	Tainan-3 mutant x Basmati-370	1992	90-105	Assam, Madhya Pradesh, Uttar Pradesh & West Bengal
10.	ADT- 41 / (JJ-92)	Selection from dwarf mutant of Basmati-370	1994	105-115	Tamil Nadu
11.	PNR-162 / (Renu)	Jaya mutant (induced) x Basmati-370	1994	90-120	Uttar Pradesh and West Bengal
12.	Birsa Dhan-107	Gora Mutant x IAC-125	1996	90-95	Bihar
13.	Gautam/ (IET-13439)	Rasi Mutant	1996	125-130	Bihar
14.	Malviya Dhan-36	Mutant of ‘Mahsuri’	1997	130-135	Uttar Pradesh
15.	Radhi / (CRM-40) / (IET-12413)	Swarnaprabha mutant	1997	120	Orissa
16.	Remanica / (MO-15) (IET 13981)	Mutant of MO1	1999	100-105	Kerala
17.	Early Samba (RNRM-7) (IET 15845)	Mutant of BPT	2000	130-135	Andhra Pradesh
18.	PNR-546 / (IET-11347)	PNR-125-2 (Induced mutant line from Pusa 150) / PNR-130-2 (Induced mutant line from Basmati -370)	2003	90-115	West Bengal
19.	Dhanu	Mutant of Ptb. 20	2006	159	Kerala
20.	Chingam	Mutant of Ptb-9 X Mutant (IR-8 X Ptb-8)	2006	95-100	Kerala
21.	Jaldi Dhan-6 / (IET 14359)	Dular Mutant/ Nagina 22 mutant	2008	65-75	Orissa
22.	CR Boro Dhan-2 / (IET 17612)	Mutant 01- China-45	2008	125-130	Orissa
23.	Malaviya Sugandh-105 (HUR-105)	Mutant of MPR 7-2	2008	130-135	Uttar Pradesh
24.	Malaviya Sugandh-4-3 (HUR-4-3)	Mutant of Lanjhi	2008	135-140	Uttar Pradesh
25.	PTB-58 / (IET-17608)	Mutant of PTB-20	2009	139	Kerala

Source: Directorate of Rice Development, Patna

Induced mutations have played a significant role for the improvement of rice by developing a large number of semi-dwarf and high yielding varieties in many countries besides important traits contributing to overall yield (Maluszynski *et al*., 1986; Baloch *et al*., 1999, 2001a, 2001b, 2002, 2003). Significant improvements through the use of induced mutations have been reported for high yield (Gaul, 1964; Goud, 1967; Reddy and Reddy, 1984; Rutger and Peterson, 1981; Chung *et al.,* 1990; Wen and Qu, 1996; Shu *et al.,* 1997; Singh, 2006; Shehata *et al.,* 2009), plant height, harvest index, leaf area index, dry matter production and grain yield per plant (Elayaraja *et al.,* 2004; Elayaraja *et al.,* 2005; Bughio *et al.,* 2007; Shehata *et al.,* 2009 and Babaei *et al.*, 2010), short stature (Rutger, 1982; Sato, 1982; Rutger, 1983; Mckenizie and Rutger, 1986; Hu, 1991; Kawai and Amano, 1991; Singh and Singh, 2003; Kumar, 2005), early maturing (Minn *et al.,* 2008 and Shehata *et al.,* 2009), 1000 grain weight and fertile tillers (Babaei *et al.,* 2010). Many physical and chemical mutagens have been used for obtaining superior mutants in rice for different traits (Hajra, 1979; Rao and Reddi, 1986; Hasan and Jagadish, 1990; Sood and Sharma, 1992; Singh *et al.*, 1998). Large numbers of mutant varieties of rice have been released in India through induced mutation or mutation breeding (Table 1).

The mutant cultivars have contributed immensely and are still contributing in augmenting the efforts of Indian plant breeders in achieving the target of food self sufficiency and strong economic growth. Mutation breeding has significantly contributed to the increased production of rice and other crops like groundnut, chickpea, mungbean, urdbean and castor in the Indian sub-continent. The success achieved with induced mutation, especially for the improvement of the major cereals of the world like rice, indicates that it is still an important tool to complement the conventional breeding technology in development of new crop varieties.

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