Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 03, Issue: 01 (JAN-FEB, 2023)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

Achievement and Prospects of Mutation Breeding

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Tropical and sub-tropical fruits are grown mainly for food and nutrition security. There is a great commercial potential for fruits in international markets for example mango, banana, citrus, papaya, litchi, and guava are major fruits consumed as fresh or as pulp or puree. Genetic improvement of tropical and sub-tropical fruits is essential for increasing fruit production, the major problem in fruit tree breeding is long juvenile phase, unavailability of suitable germplasm. Mutation is sudden heritable changes in the DNA sequence that are not derived from genetic segregation or recombination. Spontaneous mutation occurs at a very low frequency 1 in 10 lacs or 10^{-6}. In fruit crops spontaneous bud mutation are more common, called as bud sports. The occurrence of a large number of natural bud sports in citrus, mango, grapes etc. made the fruit breeders interested to breed through induced mutation. Since, mutations bring about variation, they provide the ultimate basis for evolution of new forms, varieties or species. Mutations may result into deletion, inversion, translocation of chromosome and nucleotide base substitutions. Mutation can be induced artificially with the help of various physical and chemical agents which are called mutagens. Most commonly used Physical and chemical mutagens are gamma rays and EMS (Ethyl Methane Sulphonate) respectively. Perennial nature, long juvenile phase, heterozygosity, sexual incompatibilities etc in fruit crops limits their improvement through conventional breeding system and also conventional breeding system is very time consuming so mutation breeding is emerging as new technique for rapid crop improvement.

Mutation

Mutations is defined as sudden heritable changes in the genetic material of an organism and in turn in its characters that are not derived from genetic segregation or recombination. De Vries used the word "sudden" to differentiate between subtle changes that could be explained by the normal processes of recombination. The term mutation breeding was coined to refer to the deliberate induction and development of mutant lines for crop improvement. The term has also been used in a wider sense to include the exploitation of natural as well as spontaneous mutants, and in the development of any variety possessing a known mutation from whatever source. Mutants are the individuals carrying a mutation that may be revealed using molecular means or identified by phenotypic tools. Different types of mutant can be generated using experimental mutagenesis.

General Characteristics of mutation

- Mutations are generally recessive but dominant mutations also occur.
- Mutations are generally harmful to the organism.
- Mutations are random, occur at any time and in any cell of an organisms.
- Mutations are recurrent, same mutation may occur again and again.
- Induced mutations commonly show pleiotropy, often due to mutations in closely linked genes.

- Mutations are generally harmful to the organism. In other words, most of the mutations have deleterious effects. Only about 0.1% of the induced mutations are useful in crop improvement. In majority of cases, mutant alleles have pleiotropic effects. Mutations give rise to multiple alleles of a gene.

Types of mutation

1. **Spontaneous mutation:** Spontaneously arising mutations are very rare and random events in terms of the time of their occurrence and the gene in which they occur. In this way mutant forms showing both large and small effects on the phenotype arise for all kinds of traits. Many of the mutations may be deleterious making the organism less adapted to its environment and some may even be lethal. Some may be neutral in their effects and may confer no immediate advantage, but may help to generate a wide range of useful recombinant genotypes through the subsequent generations. However, on rare occasions, some cultivars can demonstrate unstable phenotypes resulting in a portion of the plant, sometimes extending to whole branches, having different characteristics. When these branches (bud sports) are vegetatively propagated by clonal techniques, the new phenotype is generally maintained leading to a new variety, often exhibiting only one phenotypic character different from the parent. Spontaneous mutation mainly occur due to high temperature, lightning, or due to any natural calamity.
2. **Induced mutation:** Substance which induces mutations are called mutagens. They are generally grouped into two broad categories, namely chemical mutagens and physical mutagens. Traditionally, to induce mutations in crops, planting materials are exposed to physical and chemical mutagenic agents. Mutagenesis can be performed with all types of planting materials, e.g. whole plants, usually seedlings, and in vitro cultured cells. Nevertheless, the most commonly used plant material is seed. Multiple forms of plant propagules, such as bulbs, tubers, corms, shoot tip, leaf, ovules, protoplasts and rhizomes. and more recently, the induction of mutations in vegetative propagated plants is becoming more efficient as scientists take advantage of totipotency (ability of a single cell to divide and produce all of the differentiated cells in an organism to regenerate into whole plants) using single cells and other forms of in vitro cultured plant tissues. Whereas chemical mutagens are preferably used to induce point mutations, physical mutagens induce gross lesions, such as chromosomal abbreviation or rearrangements. It is noteworthy that the frequency and types of mutations are direct results of the dosage and rate of exposure or administration of the mutagen rather than its type. Tissue culture has a potential for improving effectiveness of mutation induction in several aspects. First of all, it offers a wide choice of plant material for treatment in which mutation is induced. In fact, structures that will give origin to plants are composed of a few or even of one cell. This means less risk of obtaining chimeric plants and a higher probability for mutated cells to express the mutation in the phenotype.

Mutagens

A mutagen is a natural or man-made agent that causes genetic mutation in plants or organisms, such as radiation or a chemical compound. Ionizing radiations and chemical mutagens are the most commonly employed mutagens. All sorts of planting materials, such as complete plants, generally seedlings, and in vitro cultivated cells, can be used for mutagenesis. Nonetheless, seed is the most often used plant item. Mutagens are divided into two categories: Physical mutagens and chemical mutagens.

Physical mutagens: In the past 80 years, physical mutagens, mostly ionizing radiations, has been used widely for inducing hereditary aberrations and more than 70% of mutant varieties were developed using physical mutagenesis (Mba et al., 2012). Radiation is defined as energy travelling through a distance in the form of waves or particles. These are relatively high

energy levels of electromagnetic (EM) spectrum that are capable of dislodging electrons from the nuclear orbits of the atoms that they impact upon. The impacted atoms become ions therefore, the term ionizing radiation was coined. These ionizing components of the electromagnetic include cosmic, gamma and X-rays. X-rays were the first to be used to induce mutations and gamma radiation from radioactive cobalt (60 Co) is widely used. It has high penetrating potential and is hazardous. However, it can be used for irradiating whole plants and also delicate materials, such as pollen grains.

Chemical mutagens: Chemical mutagens were found to be highly effective in inducing true gene mutations There are different chemical mutagens used to induce mutation in fruit crops namely alkylating agents such as Ethyl Methane Sulphonate (EMS), Diethyl Sulphate (DES), Ethyleneimine (EI), Ethyl Nitroso urethane (ENU), Ethyl Nitroso urea (ENH) and Azides (Heslot, 1977). As compared with physical mutagens, chemicals may give rise to relatively more gene mutations rather than to chromosomal changes. The assessment of LD50 for chemicals is determined by varying the concentration and duration of treatment, the solvent used (e.g. Dimethyl sulfoxide (DMSO), or the pH of the solution (Novak, 1991). EMS was used for inducing mutations in banana by treating shoot tips and then regenerating adventitious buds. EMS is very effective and efficient mutagen for creating of soma clonal variation in crop plants such as banana and grapes.

Mutations breeding is applicable when

- When a desirable variety has Oligo genic genetic defect.
- Desirable variability exhaust in cultivated species and germplasm.
- There is tight linkage between desirable and undesirable traits.
- Only one or two characters are to be improved in fruit crops without changing its taste.

Crop Improved through Induced Mutations

Crop improvement programs through induced mutations were started about eight to ten decades ago, immediately after the discovery of mutagenic actions of X-rays on Drosophila by Muller in the year 1927 and in barley by Stadler in the year 1928, initiated a new field of induced mutagenesis. A major breakthrough in the mutation breeding was achieved by the classic work of Gustafsson in the year 1940, in which large number of mutations in barley, especially for chlorophyll mutations and stiffness of straw were reported. At present there are many Mutant varieties of different crops released for commercial cultivation in India are, Cereals (74), Rice (42), barley (13), pearl millet (5), finger millet (7), foxtail millet(1), wheat (4), sorghum (2) pulses (57), Mung bean (15), black gram (9), chickpea (8), cowpea (10), pea (1), pigeon pea (5), French bean (1), lentil (3) Oilseeds 44 Groundnut (18), mustard (9), castor bean (4), sesame (5), soybean (7), sunflower (1), cotton (8), white jute (2), 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), opium poppy (2), spearmint (1), Mulberry (1), papaya (1), Chrysanthemum (49), rose(16), dahlia (11), portulaca (11), bougainvillea (13), wild sage (3), gladiolus(2), Hibiscus sp. (2), tuberose (2), coleus (1). The most popular mutant variety of papaya is Pusa nanha (Fig.1) and Rosica haden (Fig.2) of mango. 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.

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, with drought tolerance character, was released in 1948. In India Mutation breeding is being carried out in several national and 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.

Fig 1

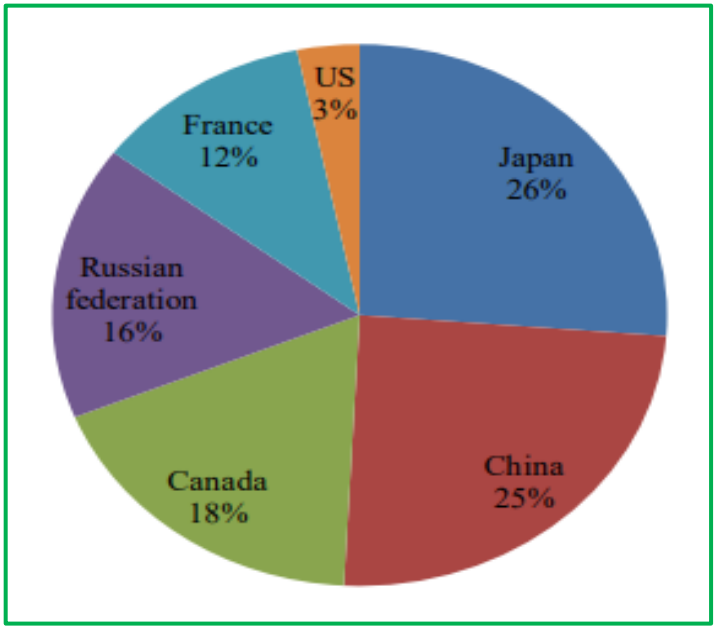
Fig 2

Limitations

- Frequency of desirable mutants is very low.
- Desirable mutations are commonly associated with undesirable side effects.
- Problem in registration of mutant variety.
- Most of the mutations are recessive.
- Many mutations are non-heritable.

Future Prospects

More research into directed mutagenesis in vegetative propagated fruit crops is needed. In fruit crops, the effects of combined mutagen treatment and recurring mutagenesis must be explored. The use of markers to detect mutations at an early stage is also required. More research is needed on in vitro mutation breeding especially for fruit crops and also for underutilized fruits crops in which favorable characters can be induced to make it more acceptable to consumers.



Country wise registered mutant varieties

Conclusion

Mutation is an important breeding tool for creating variation in fruit crops. It provides an opportunity for the improvement of traits like dwarfness, earliness, tolerance and resistance to various diseases and pests within short period of time. Mutant identification or selection at the genotypic level, using new technologies has changed the way, mutations are now used in genetics and breeding in Fruit crops. In vitro culture combined with induced mutation had been proven to speed up the breeding program to produce genetic variations or for multiplication. It also help in development of commercial varieties in achieving the target of nutritional security.

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