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Mutation breeding in vegetable crops: A review

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Abstract
Mutation breeding generally plays a very important role in vegetable crops. Among the different present approaches, mutagenesis and mutation breeding and the isolation of improved or novel phenotypes in conjunction with conventional breeding programmes can result in mutant varieties endowed with new and desirable variation of agrometrical traits. Induced mutations and its related technologies play very well in this ground and this overall strategy helps to trace the crop genetic diversity along with its biodiversity maintenance. Such induced mutagenesis, a crucial step in vegetable crop improvement programme, is now successful in application due to the advancement and incorporation of large-scale selection techniques, micro propagation and other *in vitro* culture methods, molecular biology tools and techniques in modern crop breeding performance. Molecular mutation breeding will significantly increase both the efficiency and efficacy of mutation techniques in crop breeding. Thus, mutation assisted plant breeding will play a crucial role in the generation of 'designer crop varieties'. This paper provides a comprehensive overview of the various techniques and workflows available to researchers today in the field of mutation breeding, and how these tools complement the ones already used in traditional breeding.

Keywords: Mutagenesis, EMS, gamma rays, vegetable crops, mutants, mutation

Introduction
Vegetable breeding categorized into three sub-types as mutation breeding, recombination breeding and transgenic breeding. In case of mutation breeding, the basic fundamental and the unique feature is the generation of new mutated alleles. Hugo de Vries in 1900 used the term "mutation" to describe phenotypic changes that are inheritable. Utilization of induced mutations for crop improvement is known as mutation breeding. During the 1950s, induced mutagenesis was widely pursued in the US, Europe, Japan and China. In India, Swaminathan and his team at the Indian Agricultural Research Institute, New Delhi initiated a major programme on mutagenesis in crop plants. The key steps include analysis of difference in the sensitivity of different genotypes and plant tissues to different mutations often measured using lethal doses (LD).

Mutations may induce both qualitative as well as quantitative variation comparatively in a shorter period of time by altering alleles at known loci as well as at previously unknown loci, besides altering linkage groups (Konzak *et al*. 1977) [9]. Induced mutagenesis has been used to obtain direct mutants or by using these mutants in hybridization (Ahloowalia *et al*., 2004) [1] to overcome yield plateaus and generate desirable horticultural traits. A study of induced variability for chlorophyll and viable morphological mutations in the M2 generation was the most dependable tool to utilize useful mutations for efficient crop improvement (Kumar *et al*., 2007) [12]. With the new fangled impulse in plant mutation research from basic mutational studies to modern reverse genetics, breeders at present are able to exploit mutation techniques more sophisticatedly than before.

History of vegetable mutation
The discovery of X-rays by Wilhelm Roentgen in 1895 led to the application of X-rays for inducing mutations in *Drosophila melanogaster* by Muller (1927) and in barley by Stadler (1928). This technique later became the most important tool for locating genes on chromosomes, studying gene structure, gene expression and regulation and for exploring genomes (Solanki *et al*. 2011) [22]. *Nicotiana tabacum* was the first crop in which the first commercial mutant variety "chlorine type" was induced. In general, ionizing radiations such as X-rays and gamma rays are preferred because of their easy application, good penetration, reproducibility, high mutation frequency and less disposal problem. All chemical mutagens

react with DNA by alkylating the phosphate groups and also the purine and pyrimidine bases. The dose of a mutagen applied is an important consideration in any mutagenesis programme. The lethal dose-50 (LD50) gives an idea about the optimum dose of the mutagens. Optimum dose produces the maximum mutations with minimum hazards. An overdose of mutagens will kill too many plants, while low dose will produce too less mutation spectrum and frequency. The mutagenic dose mainly depends upon the concentration, duration of treatment and temperature during the treatment. Pre-soaking, pH of solution, metallic ions, carrier agents, post washing, post drying and storage of seeds are the modifying factors for the mutagenic effect (Solanki *et al*. 2011) [22]. The physical and chemical mutagens cause three types of effects, i.e. physiological damage, gene mutations and chromosomal aberrations. Gene and chromosomal mutations may be transferred from M_1 to succeeding generations; however, physiological effects are generally restricted to the M_1 generation. Gene mutations occur spontaneously as errors during DNA replication. Mostly these errors are repaired; however, some may pass to the subsequent cell division and establish in plant offspring. Artificial induction of mutations by ionizing radiation dates back to the beginning of the twentieth century. But it took many years to establish that such changes could be beneficial for plant breeding. Hence, crop improvement using classical mutagenesis is now well standardized and as a result, new methods of radiation treatment, as well as chemical agents with mutagenic properties are serving as invaluable tools for augmentation of the genetic variation in crops to circumvent bottleneck conditions.

Chlorophyll mutations, effectiveness and efficiency of mutagens

Chlorophyll mutations, in general, are considered as the measure to access the effectiveness of treatments of various mutagens. Gustafsson (1940) grouped chlorophyll mutations into albina, xantha, viridis, chlorina, striata, tigrina and maculata classes. Numerous authors have so for reported the incidence of different types of chlorophyll mutations such as albina, xantha, chlorina, viridis, virescent, tigrina, etc. in M_2 generation following mutagenic treatments (Girija and Dhanavel 2013) [4]. Singh and Singh (2007) [21] reported four different types of chlorophyll mutations, i.e. albina, xantha, chlorina and viridis in mungbean. They reported that the frequency of chlorophyll mutations was higher in the population treated with EMS.

Mutagenic effectiveness defines mutagen dose to the mutational events, while mutagenic efficiency is the production of desirable changes that are free from associations with undesirable genetic alterations. This is generally measured by the proportion of the mutation frequency in relation to damages associated to mutagenic treatments such as height reduction, chromosome breakages, sterility, lethality, etc. (Sikder *et al*. 2013) [20]. The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and quantify the frequency as well as the pattern of changes in different selected plants by mutagens. The ability of these mutagens to enter the cell of living organisms and to interact with DNA produces the general toxic effects. Thus, their effects are mainly due to the direct interaction between the mutagen and the DNA molecules. Mutagenic effectiveness and efficiency were found to be increased at lower doses of the mutagens. Chemical mutagens, on the whole, have been found to be more efficient than gamma rays. The values of efficiency provide an idea of the extent and type of damage caused by the mutagen in question.

Tilling: A best screening tool for mutant plant population

The power of TILLING was first demonstrated in model systems such as *Arabidopsis* and *Drosophila* (Mc. Callum *et al*., 2000) [13] where it was shown that single mutations in specific genes could be identified. TILLING has later been successfully applied to a number of plant systems including barley, wheat, maize, rice, oat, pea, and soybean. Thus, this technology provides the breeders with a new and sophisticated tool for crop improvement.

In recent years, the availability of genomic sequences from many plant species and the development of a wide array of molecular-genetic technologies have enhanced our ability to detect or engineer such variation at specific genetic loci (reverse genetics), greatly expanding our capacity for both probing gene function and genetic engineering. McCallum *et al*. (2000) [13] have introduced a new reverse genetic strategy that combines the high density of point mutations provided by traditional chemical mutagenesis with rapid mutational screening to discover induced lesions. TILLING (Targeting Induced Local Lesions IN Genomes) combines chemical mutagenesis (Koornneef *et al*. 1982) [10] with a sensitive mutation detection instrument. The TILLING strategy utilizes traditional mutagenesis followed by high throughput mutation discovery.

Minoia *et al*., 2010 [15] developed a new mutant genetic resource for tomato crop improvement by TILLING technology. The general applicability of TILLING makes it appropriate for genetic modification of vegetable crops. After mutagenic treatment with ethyl methane sulfonate (EMS), the resulting M1 plants are self-fertilized to get the M2 individuals which are used to prepare DNA samples for mutational screening.

Effect of physical mutagens in vegetable crops

The goal in mutagenesis breeding is to cause maximal genomic variation with a minimum decrease in viability. Among the radiation-based methods, γ-ray and fast neutron bombardment now supersedes X-ray in most applications. Of these, γ-ray bombardment is less destructive causing point mutations and small deletions whereas fast neutron bombardment causes translocations, chromosome losses, and large deletions.

Kangarasu *et al*., 2014 [18] used different doses of gamma rays i.e. 15, 20 and 25 kR to induce flower colour and seed mutants in M_2 generation mutation in dormant seeds of *Phaseolus vulgaris* L. cv. Waghya. Mutants with different flower colour and altered size, shape and seed coat colour were obtained by 20 kR dose. Micro-mutations were also scored by Mejri *et al*. 2012 for percentage of germinated seeds, pod length and photosynthetic pigment contents in faba bean.

The variation in DNA profile in responses to gamma irradiation treatments was detected by RAPD -PCR technique in variety Sabahia of okra [*Abelmoschus esculentus* (L.) Moench] (Hegazi and Hamildeldin, 2013) [7]. The relatively high doses of gamma irradiation (400 and 500 Gy) induced more changes in genomic DNA pattern than the low dose (300 Gy). Hassan and Halem (2014) [6] studied the effectiveness of different doses of gamma rays to induce new genetic variability in some agronomic traits of canola (*Brassica napus* L.).

Nouri and tavassoli, 2012 used one of the successful and experimented methods of mutation and irradiation techniques with use of gamma rays to identify the intensity of radiation described for understanding the changes desired phenotypic, morphological and physiological on pinto bean seeds of Khomein cultivar.

Aney (2014) [2] also studies the effect of gamma irradiation on yield attributing characters in two varieties of pea (*Pisum sativum* L.). Both the varieties showed dose dependent decrease in most of the yield attributing characters, but the genotype of var. *arvense* is observed more sensitive to the doses of gamma irradiation than var. *hortense*.

Effect of chemical mutagens in vegetable crops

In any mutation breeding program, selection of an effective and efficient mutagen is very essential to produce high frequency of desirable mutation. Shah *et al*. (2015) [18] uses different doses of ethyl methyl sulfonate (EMS) in Chinese Long cultivar of cucumber to induce variability. In all experiments with increasing EMS concentration, germination percent, index, and rate were decreased.

Induced mutants of cauliflower can also be screened for drought and salt tolerance through N-nitroso-N-ethyl urea (NEU) and N-nitroso-N- methyl urea (NMU) (Hadi and Fuller, 2013). Shalaby and Banna (2013) developed an invitro technique suitable for mutation induction on tomato and characterize them by RAPD and SSR markers as well as horticultural characteristics. Mutagenized population also serve as a resource for high throughput reverse genetic studies to screen for point mutations in specific regions of targeted genes (Reddaiah *et al*., 2014) [17].

Elangovan and Pavadai (2015) [3] conducted an experiment to determine the effect of different concentration of ethyl methane sulphonate (EMS) and diethyl sulphate (DES). The highest mean value for all parameters was recorded in 0.5% of EMS and 0.4% of DES treatment than the other treatments. The maximum 100 seed weight was recorded in 0.4% of DES treatment.

Combined effect of both physical and chemical mutagens in vegetable crops

Combined effect of induced mutation has become an effective tool to improve vegetable crops through creation of variability in vegetable crops.

Cowpea being a self pollinated vegetable crop has very limited genetic variability, therefore induced mutation can provide additional source of mutation in recent plant breeding programs. Kumar and Verma (2011) [11] has made an attempt to study the mutagenic effects of gamma rays and sodium azide on the meiotic cells of *Vigna unguiculata*. Chromosome aberrations like unorientations, multivalent, laggards, bridges and precocious movements etc. were noticed in mutagen treated population.

Singh *et al*. (2011) [22] conducted experiment on seeds of three genotypes of okra viz., Parbhani Kranti, Hisar Unnat and Satdhari treated with gamma rays (15, 30, 45 and 60 kR) and EMS (0.25, 0.50, 0.75 and 1.00%). Higher doses of EMS and gamma rays had deleterious effects on seed germination, plant survival, seedling height and pollen and ovule fertility.

Conclusions and future perspective

Globally, food security has witnessed a major deterioration in the past few years; food costs are mounting brusquely and poor people are threatened with serious malnutrition. With population explosion, the demand for food is enormously on the rise, while natural resources are depleting with every passing day. Erratic rainfalls, impetuous drought conditions, excessive floods, etc., often related to climate change, further exacerbate the miseries by deteriorating the crop production conditions. Under these circumstances, it is imperative that the yield potential of the crop plants has to be significantly increased to combat the aggravating food security situation. Induced mutations have the ability to increase the rate of domestication of many vegetable crops that may be potentially useful as a source of food, forage and industrial raw materials. It is striking that a huge number of mutant varieties have been developed and widely cultivated in developing countries, hence greatly improving their food security. In recent years, induced mutations, as a tool, have been gaining momentum in the field of plant molecular biology to identify, isolate and study the structure and function of such genes which are actually of imperative use in breeding studies. The fundamental knowledge of genes which control core agronomical and quality traits is vital for plant breeders to frame appropriate strategies and ingeniously implement them in breeding programmes for prosperous results.

Recently, mutation techniques have also been integrated with other molecular technologies, such as molecular marker or high-throughput mutation-screening techniques, and are becoming more powerful and effective in breeding crop varieties. In addition, the reverse-genetic strategies like targeting induced local lesions in genomes (TILLING) that target lesions to specific genes are anticipated to speed up the process of gene function analysis and the efficiency of mutation breeding for better crop future.

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