Review

Genetic improvement of fruit crops through in vitro mutagenesis

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 Received: 30.05.2025/Accepted: 03.07.2025

ABSTRACT

Traditional plant breeding methods often face significant hurdles when attempting to introduce new genetic variations into fruit crops, largely due to existing genetic limitations. This review explores how mutation breeding, particularly when combined with in vitro culture techniques, offers a highly efficient and rapid pathway to genetic improvement. Beyond merely creating new mutants, this integrated approach excels at enhancing specific traits within already superior varieties, breaking undesirable genetic linkages and expanding overall variability. The revolutionary impact of in vitro methods on mutation induction are delved into, discussing how they overcome challenges like material availability and chimera formation while facilitating the rapid isolation and cloning of desired genetic variants. The review examines various physical and chemical mutagens used, detailing their modes of action and the critical factors influencing mutagenic sensitivity, such as dose, treatment duration, genotype and explant type. Furthermore, the diverse and beneficial effects observed in fruit crops are highlighted, ranging from cytological and morphological changes to significant improvements in vegetative traits (like germination and vigour) and reproductive characteristics (including early flowering, increased fruit yield and enhanced quality). This comprehensive overview underscores the established and growing importance of in vitro mutagenesis as a sophisticated and indispensable tool for modern fruit crop breeders, paving the way for the development of more productive and resilient varieties worldwide.

Keywords: Fruit crop improvement; in vitro mutagenesis; mutation breeding; plant growth regulation; genetic variability; tissue culture; mutagenic agents; crop enhancement

INTRODUCTION

Improving fruit crops through traditional plant breeding methods often faces limitations due to the existing genetic material. In such cases, mutation stands out as the sole way to introduce new variations quickly. When mutation breeding is combined with tissue culture, it proves even more effective than conventional breeding techniques. Beyond just creating valuable new mutants, mutation breeding offers several advantages. It's particularly useful for enhancing a specific trait in an already well-adapted and desirable variety, for breaking strong genetic linkages to create rare new combinations and for expanding the range of variability in quantitative characteristics (Predieri 2001). In fruit crops specifically, mutagenesis has already been instrumental in developing many beneficial traits, including changes in plant size, bloom time, fruit ripening, fruit colour, improved quality, self-compatibility, natural fruit thinning and increased resistance to diseases (Maluszynski et al 1995, Kaushal et al 2004). Most of the genetic diversity currently used in breeding programmes has occurred naturally and is found in collections of older and newer cultivars, local varieties and other genetic types. However, when these existing genetic resources don't provide the specific combinations breeders are looking for, it becomes necessary to explore other sources of variation. Since natural mutations happen very rarely, induced mutation techniques offer a fast way to create and increase variability within crop species. According to Brock (1976), these induced genetic changes are a more efficient source of genetic variability than relying solely on natural gene pools. The importance of artificially induced mutations in cultivated plants has been recognized by many plant breeders globally for several

years. Today, mutation breeding is an accepted method available to breeders for crop improvement. This is especially true for crops that reproduce vegetatively, as it allows for the alteration of just one or a few traits while preserving the overall superior characteristics of an existing cultivar.

In vitro mutagenesis

The development of effective in vitro culture methods has revolutionized how we use mutation techniques to improve both seed and vegetatively propagated crops. For many crops that reproduce vegetatively, combining mutation induction with in vitro culture might be the only efficient way to achieve plant improvement (Novak 1991). Several researchers have already explored using in vitro produced tissues to establish optimal treatment parameters for mutation induction (de Guzman et al 1980, Omar et al 1989, Novak et al 1990). Working with in vitro tissues helps overcome common challenges like the limited availability of plant material, issues with experimental reproducibility and the poor uptake of chemical mutagens. By repeatedly micro-propagating plants through axillary bud stimulation after mutagenic treatments, it becomes easier to isolate somatic mutants more frequently. This approach also saves significant space and time compared to traditional somatic mutagenesis methods (Donini and Micke 1984). Furthermore, inducing mutations in vitro can boost the production of isogenic lines derived from mutated sections, individual cells or even protoplasts (Kameya 1975). In essence, tissue culture significantly increases the efficiency of mutagenic treatments for inducing variation, allows for handling large populations, facilitates the use of direct selection methods and enables the rapid cloning of desired variants (Jain 2005). In vitro techniques are also becoming increasingly vital in mutation breeding to prevent or limit the formation of chimeras (Broetjes et al 1976). Plants or shoots that grow from adventitious buds typically originate from just one or a few totipotent cells (Broertjes and van Harten 1978). After applying mutagens, some of these new buds might become chimeric, meaning they contain both mutated and non-mutated sections. To isolate the purely mutated sector, multiple cycles of micro-propagation can be performed (Maluszynski et al 1995).

Mutagens

To induce mutational changes in plant material, mutation breeders can choose from two main categories of mutagenic agents: physical mutagens (like X-rays and gamma rays) and chemical mutagens (such as ethyl methane sulphonate, methyl methane sulphonate and sodium azide). While some researchers suggest that mutations result in both chromosome breakage and gene mutations (Dubinin and Soyfer 1969), often, 'true mutations' refer specifically to changes at the gene level. Chemical mutagens work by inducing various types of mutations within the plant's genome. These chemicals interact directly with the DNA bases, leading to chromosome breaks or subtle point mutations. They are classified into different groups based on their structure and how they act.

The most commonly used chemical mutagens are alkylating agents. These substances react with DNA by adding alkyl groups to its phosphate groups and to the purine and pyrimidine bases. Among them, ethyl methane sulphonate (EMS) is highly favoured. Loveless (1958) was the first to demonstrate EMS's mutagenic effect and Freese (1963) later categorized it as a mono-functional alkylating agent with an active ethyl group. EMS is known for producing point mutations with minimal damage to the chromosomes (Amano and Smith 1965). Chemical mutagens have been successfully used for in vitro mutagenesis in several fruit crops, including apple (Fu et al 1995), banana (Omar et al 1989) and cherry (Yang and Schmidth 1994). For instance, Liu et al (2008) investigated the optimal EMS concentration for inducing salt tolerance mutations in strawberry callus and leaf tissues under in vitro culture conditions.

Plant material for in vitro mutagenesis

Mutations are singular cellular events that can, in theory, be induced in any cell or tissue within a plant (Broertjes and van Harten 1978). However, not all cells and tissues respond identically when exposed to mutagens. Many scientists have irradiated shoot tips of plants like banana (de Guzman 1980, Novak et al 1990, Bhagwat and Duncan 1998) and grapevine (Charbaji and Nabulsi 1999) with gamma rays to induce in vitro mutations. Microshoots have also been utilized in studies involving cherry (Predieri and Zimmerman 2001).

Furthermore, leaves have served as the plant material for inducing in vitro mutagenesis in grapevine (Kuksova et al 1997) and cherry (Yang and Schimdt 1994). Shen et al (1990) conducted experiments where aseptically cultured shoots and petioles from the first and second leaves below the shoot tip of Chinese gooseberry were subjected to gamma irradiation.

Method of treatment

Physical mutagen treatment: For physical mutagens, a common approach is to treat larger plant parts with direct irradiation before they're even removed for tissue culture. After this initial treatment, standard explanation and in vitro culture procedures follow. For example, Shen et al (1990) irradiated aseptically cultured Chinese gooseberry shoots that were growing on a half-strength MS medium with gamma rays. Similarly, in the Highgate banana variety, dissected apices were cultured in a liquid initiation medium for four weeks before being exposed to gamma irradiation (Bhagwat and Duncan 1998). Right after irradiation, these explants were moved to a proliferation medium.

Chemical mutagen treatment: When using chemical mutagens, there are two main ways to apply them. These can be added in precise amounts to an already autoclaved (sterilized) growth medium to reach the desired final concentration or the plant explants can be gently agitated directly in an aqueous solution of the mutagen (Singh et al 2000). If mutagens are being incorporated into the medium, remember they need to be filter-sterilized first, not autoclaved. That's because high temperatures during autoclaving can reduce their effectiveness. All mutagens can be diluted in water or a portion of the culture medium before these are added to the sterilized medium. After the treatment is complete, it's crucial to either change the medium or rinse the explants thoroughly and then transfer them to a fresh, mutagen-free medium. For instance, Omar et al (1989) treated banana shoot-tips by dipping them into aqueous solutions of filter-sterilized EMS at various concentrations for three hours, maintaining a constant temperature. Afterward, these shoot tips were carefully washed with sterile water and then placed on a nutrient medium for culturing. Fu et al (1995) followed a similar procedure for apple leaf pieces and shoot segments.

Mutagenic sensitivity: Before the actual mutagen treatment, the tissue is subjected to sensitivity trials to find out the effective dose levels. According to Sparrow et al (1967), mutagenic sensitivity depends mainly on the nuclear volume (greater the DNA content, the more sensitive), the number of chromosomes (plants with fewer chromosomes, given a certain nuclear volume, are more sensitive than plants with more, smaller chromosomes) and the ploidy level (the higher it is, the less sensitive). At the same time, the genetic factors, climatic and other environmental conditions before and after treatment of the plant part as well as the stage of development of shoot or root also seem to be of importance. The response of cells of higher plants to physical and chemical mutagens is influenced to significant extent by numerous biological, environmental and chemical factors (Anon 1977b).

Choice of doses: The length of time samples are exposed to radiation depends on the desired dose and the rate at which that dose is delivered. For gamma rays, the dose is measured in Grays (Gy), where 1 Gy equals 100 R. The dose rate, on the other hand, refers to how quickly a specific amount of radiation is administered. When it comes to chemical mutagens, the dose is determined by combining the chemical's concentration with the treatment's duration. Generally, the mutagen dose that leads to a 50 per cent reduction in plant growth is used as an indicator of how sensitive the plant is to the radiation (Novak et al 1990). For instance, Arunyanart and Soontronyatara (2002) observed a 50 per cent survival rate in tissue-cultured plantlets after treating them with either acute gamma rays at doses of 0, 2, 3, 4, 5, or 6 kr or with X-ray treatment.

Time of treatment: Beyond just the dose, the effectiveness of mutagens also depends heavily on the treatment duration, especially with chemical mutagens. For instance, Fu et al (1995) treated tiny apple leaf pieces (0.5 cm^2) from the Freedom cultivar with varying concentrations of EMS for either 1.5 or 3.0 h. Their findings clearly showed that the longer the treatment, the lower the survival rate of the explants. Specifically, at a 0.2 per cent EMS concentration, the survival of leaf explants dropped significantly from 88.9 to 55.8 per cent when the treatment time increased from 40 to 80 minutes. Based on these results, they concluded that the LD_{50} (lethal dose 50%) for this specific scenario was 0.2 per cent EMS applied for 80 minutes.

Genotypes

An organism's sensitivity to a mutagen isn't just about the mutagen itself; it's also shaped by several other factors. These include its chromatin content (Sparrow et al 1967), its genetic makeup (Blixt 1968), and its physiological state both before, during and after treatment (Brock 1965). For example, Predieri and Zimmerman (2001) exposed in vitro shoots from six different pear cultivars to gamma irradiation at a dose of 3.5 Gy. Micro-cuttings taken from these irradiated shoots were then rooted and planted in the field. They observed variations in fruit characteristics like the

degree of russeting, fruit shape and size. The frequency of these observed fruit variations changed depending on the cultivar, ranging from 0.81 per cent in Doyenne d'Hiver to 3.64 per cent in Passe Crassane.

Explants

Different types of plant tissues can show varying levels of sensitivity to mutagens. For example, Bhagwat and Duncan (1998) exposed two kinds of banana Highgate explants to gamma irradiation: Type I (dissected apices) and Type II (corms from in vitro derived shoots). They measured radio-sensitivity by counting how many explants survived. Eight weeks after irradiation, the survival rate for Type I explants varied from 100 per cent in the untreated control group down to 38.2 per cent at 5.0 kr. Generally, survival remained quite high (over 75%) for treatments between 1.0 and 4.0 kr. For Type II explants, survival ranged from 100 per cent in the control to 64.5 per cent at the 4.0 kr treatment. However, at 5.0 kr, only 29.4 per cent of these explants survived.

Mutation frequency

The core goal of any mutation breeding programme is to boost both the frequency of mutations and the range of variations produced. Many researchers, for example Zareen and Devi (1995), have used chlorophyll mutation frequencies as a key indicator of mutagenic effectiveness. Generally, it is seen that the more significant the mutagen dosage, the higher the mutation frequency.

Mutagen effects

Mutations caused by the physical and chemical mutagens effects may manifest itself in several morphological, cytological or biochemical variations.

Cytological effects: Some effects of mutagenic treatments, particularly significant changes like chromosome mutations, can actually be observed and recorded using cytological methods during both mitosis and meiosis (Anon 1977a). For instance, in the grapevine cultivar Podarokmagaracha, gamma irradiation (at doses from 5-100 Gy) of leaf explants has been found to lead to an increase in the tetraploid plant formation. This occurred in 7 per cent of primary calluses and 7.6 per cent of embryogenic calluses and researchers also found some aneuploid plants (Kuksova et al 1997).

Morphological changes: Morphological changes can often be seen and measured either on a specific plant organ or as a response across the entire organism (Sparrow 1961). The most significant of these effects are usually the death of the treated material or a slowdown in growth. For instance, Novak et al (1990) exposed in vitro cultured banana shoot tips to gamma rays. They successfully produced variants that displayed noticeable changes in their plant stature, leaf shape and colour and how many suckers they produced. The frequency of these variations ranged from 3 to 40 per cent, directly depending on the radiation dose used. Similarly, Fu et al (1995) treated in vitro derived apple shoots. They cultured these on an MS medium containing filter-sterilized EMS (0.2%) for three hours, then transferred them to a mutagen-free medium. This process resulted in variants showing distinct traits like forked leaves and leaves growing in opposite pairs.

Some reports suggest that even low doses of mutagens can actually promote growth in in vitro cultured plantlets. For example, Charbaji and Nabulsi (1999) exposed shoot tips and single node explants from two grapevine rootstocks (R.99 and 3309) and two varieties (Helwani and Cabernet Franc), all cultured on DSDI media, to gamma irradiation. They found that low doses, specifically ranging from 5 to 7 Gy, led to an increase in root lenght and number and shoot length and noted an increase in dry weight and number of leaves. In a different study, grey-red coloured and flat-shaped ray florets were similarly treated with gamma irradiation at doses of 0.5 and 1.0 Gy.

Vegetative characteristics: Kumar and Mishra (2004) observed that in okra (*Abelmoschus esculentus*), the germination rate generally fell as doses or concentrations of gamma rays and EMS increased. Similarly, Dhakshanamoorthy et al (2010) found that higher doses or concentrations of gamma rays and EMS drastically shortened the roots, shoots and overall seedling length, also reducing the vigour index in *Jatropha curcas*. Regarding plant height, EMS treatments led to noticeable variability in *Capsicum annum* (Jabeen and Mirza 2004). Jamil and Khan (2002) specifically noted that radiation doses of 5 and 10 Krad caused a slight reduction in plant height, while other doses had no significant impact.

Reproductive characteristics: Researchers identified an early-flowering plant within a population of Grand Naine bananas that had been regenerated from shoot-tips exposed to 60 Gy of gamma irradiation (Novak et al 1990). This particular plant, named GN-

60Gy/A, grew vigorously and started flowering in just nine months, significantly faster than the non-irradiated control plants, which took 15 months. This accelerated flowering time remained consistent across its second and third generations as well. Similarly, in vitro mutation induction using gamma irradiation led to the selection of Novaria, an early-flowering mutant of Cavendish banana (Mak et al 1999). Beyond flowering time, gamma ray irradiation also resulted in increased fruit yield and larger bunch sizes in bananas (Smith et al 2006).

CONCLUSION

Traditional plant breeding often faces limitations in enhancing fruit crops. In vitro mutagenesis offers a powerful and rapid alternative, efficiently introducing genetic variations and specific trait improvements that are difficult to achieve conventionally. By integrating with tissue culture, this method overcomes challenges like material availability and chimera formation, allowing for precise control and rapid selection of desired variants. Mutagens, whether physical or chemical, induce beneficial changes, from improved plant stature and blooming time to enhanced fruit quality and disease resistance. While sensitivity varies by dose, genotype and explant, this technique consistently leads to significant advancements in germination, vigour and overall yield. Ultimately, in vitro mutagenesis stands as an indispensable tool for modern fruit crop breeding, driving the development of more productive and resilient varieties.

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