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IN VITRO MUTATION IN HORTICULTURAL CROPS- A REVIEW

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ABSTRACT

***In vitro* mutation can create novel and unique variations when the natural variability does not provide the genes for the desired trait. The creation of variability is the starting point in any breeding programme of an agronomically accepted cultivar rather than a genetic stock of no direct commercial value with many undesirable attributes. In *in vitro* mutation, the LD_{50} value for shoot tip explants was 2.00 kR gamma rays and 750 mM EMS. Similarly, the LD_{50} value for callus culture was 1.0 kR gamma rays and 200 mM EMS. The callus derived *in vitro* mutants exerted negative genotypic and phenotypic correlation was expressed by forskolin content. The work on *in vitro* mutation in different horticultural crops has been reviewed in this article.**

Key words: Mutation, Horticultural crops.

In vitro mutation can create novel and unique variations when the natural variability does not provide the genes for the desired trait (Chopra and Sharma, 1991). *In vitro* techniques are also becoming more and more important for use in mutation breeding to prevent or restrict chimera formation. The application of plant tissue culture for the induction of heritable changes has now been demonstrated in a range of crops. When explants are grown *in vitro*, the tissue culture environment itself appears to upset the normal cell division and chromosome distribution. In many vegetatively propagated crops, mutation induction in combination with *in vitro* culture techniques may be the only effective method for crop improvement (Novak, 1991).

Following mutagen applications, some of the new buds become chimeric with mutated and non-mutated sectors. Separation of mutated sector from the non-mutated sector can be achieved through a number of cycles of micropropagation to obtain the mutated sector alone (Maluszynski *et al.,* 1995). Predieri (2001) opined that *in vitro* mutation induction has higher potential with positive achievement in crop improvement programmes.

1. Mutagens

For the induction of mutational events in plant material, the mutation breeder can choose between two groups of mutagenic agents namely, physical (X-rays,
Gamma rays etc.) and chemical (Ethyl Methanesulfonate, Methyl Methanesulfonate, Sodium azide etc,). The former have been used for many decades whereas, the use of chemicals started in 1940's.

Irradiation in combination with *in vitro* culture has proved to be a valuable method of producing desirable variations and rapid propagation. Research on radiation induced *in vitro* mutations has been carried out on potato (Ahloowalia, 1990), banana (Bhagwat and Duncan, 1998 and Prabhakaran, 2001), cassava (Safo-Kantanka and Owusu-Nipah, 1993), yam (Klu, 1993), gerbera (Laneri *et al.,* 1990), lemongrass (Nayak *et al.,* 1997) and garlic (Malpathak and David, 1990). Ram *et al.* (1994) reported that sodium azide was best over X-rays and EMS. Application of radiation has been most frequently used for mutation induction resulting in the direct development of mutant varieties (89 % of the total mutant varieties) and gamma ray treatment was employed for the development of 64 per cent of the radiation induced mutant varieties (Nichterlein *et al.,* 2000).

2. Plant materials used for *in vitro* mutagenesis

Latado *et al.* (2004) irradiated pedicels of chrysanthemum with 8 Gy of gamma rays and

obtained a mutation rate of 6.67 per cent. In banana, embryogenic calli, raised from male flower buds were exposed to gamma rays (Kulkarni *et al.,* 1997). *In vitro* derived callus has also been used in *Cymbopogon flexuosus* (Nayak *et al.,* 1997).

In chrysanthemum, Latado *et al.* (2004) affirmed that sensitivity of pedicels to EMS revealed that LD_{50} was close to 0.82 per cent (v/v). Paramesh and Chowdhury (2005) stated that *in vitro* shootlets of carnation cv. IIHRS-1 were subjected to irradiation with gamma rays (20, 40, 60 and 80 Gy). The results indicated that survival percentage decreased with increasing dose of gamma irradiation. Dao *et al.* (2006) developed protocol for *in vitro* mutagenesis in chrysanthemum and found that the lethal dose was about 5.0 kR for calli irradiation. After 10 days of irradiation, the calli of the control and those treated with 1.0 and 3.0 kR gamma rays were green and compact, while those treated with higher dosages turned brown and friable. In control and 1.0 kR dose, the regeneration rate reached 97.5 and 90 per cent respectively, while it was 75 and 20 per cent in 3.0 kR and 5.0 kR doses respectively.

In *Coleus forskohlii*, Velmurugan (2007) and Velmurugan *et al.* (2008) opined that the LD_{50} value for shoot tip explants was 2.00 kR gamma rays and 750 mM EMS. Similarly, the LD_{50} value for callus culture was 1.0 kR gamma rays and 200 mM EMS.

3. Method of treatment

Walther and Sauer (1990) investigated the effect of split versus acute X-ray doses on *in vitro* derived micro-shoots of *Gerbera jamesonii.* Splitting of doses between 10 Gy and 50 Gy into two or three fractions separated for period of four hours and each recovery led to considerable rise in shoot production. In banana variety High gate, dissected apices cultured in liquid initiation medium for four weeks were subjected to gamma irradiation (Bhagwat and Duncan, 1998).

4. Mutagenic sensitivity

Jerzy and Lubomski (1992) irradiated *ex vitro* derived leaf explants of gerbera with 0.5 to 2.5 kR doses of gamma rays. They observed that adventitious shoot formation was dependent on the dose of irradiation. Doses of 2.0 and 2.5 kR drastically reduced the regeneration ability of leaf explants, but even with the highest dose, some shoots were formed. They opined that the optimum dose of the radiation ranged from 1.0 to 2.5 kR.

Ibrahim *et al.* (1998) analysed the radiosensitivity of three *Rosa hybrida* L. genotypes by irradiating leaf explants with increasing doses X-rays between 2.5 and 10 kR. The results revealed a decreasing rate of regeneration of buds (47 % and 0 %) was noticed at 2.5 and 10 kR respectively. In banana cv. Grand Naine, the growth of embryogenic cells abridged with an increasing dose upto 30 Gy and a dose of 40 Gy and beyond was observed to be completely lethal. The cell weight was higher than the control, but reduced with an increasing dose of irradiation. The gain in fresh weight and dry weight of cells were noticed at lower irradiation doses (Kulkarni *et al.*, 2004).

5. Choice of doses

The duration of exposure of the samples to radiations depend on the required dose and dose rate. Gamma ray dose is expressed as kilo Roentgen (kR) (1 kR = 1000 rads). Prabhakaran (2001) recorded the LD_{50} value for Rasthali (30 Gy) and Red banana (40 Gy). While the LD_{50} concentration for EMS was 0.3 and 0.4 per cent for Rasthali and Red banana respectively. The number of days taken for greening of explants, days to first leaf emergence and days taken for root initiation under *in vitro* condition were found to increase with increase in dosage of irradiation and concentration of EMS. In black pepper, higher doses of gamma rays (40 and 50 Gy) produced brown friable calli. But the cultivar Kalluvally withstood a higher dose of gamma irradiation bestowed cream nodular calli even at 40 Gy (Shylaja and Nair, 2003).

Similarly in Dendrobium hybrids, Shobhana and Rajeevan (2004) found that the protocorms produced from the hybrid seeds obtained from the cross Emma white x Banyat pink were subjected to irradiation using gamma rays (10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy and 60 Gy) under *in vitro* condition. The experimental results revealed that time taken for differentiation of leaf as well as for shoot and root formation was more in the irradiated cultures; irradiation reduced the height of plants and produced broader leaves with dark green colour.

Datta *et al.* (2005) stated that *in vitro* cultures of Ray florets of *Chrysanthemum morifolium* cvs Flirt, Puja, Maghi and Sunil were treated with gamma

rays (500 and 1000 rad). The research findings revealed that frequency of direct shoot regeneration decreased in gamma ray treated florets. Radiation effect was also found on plant regeneration from gamma ray treated floret explants and also on plant height, size of leaf and flower. Tejaswini *et al.* (2006) opined that survival percentage and percentage of explants showing growth was reduced with the increased concentration of EMS in media. On the contrary, the percentage of explants exhibiting abnormal response increased with increased concentration of EMS fortified media.

6. Choice of genotype

Predieri and Zimmerman (2001) subjected *in vitro* shoots of six pear cultivars to gamma irradiation at a dose of 3.5 kR. Micro cuttings from the irradiated shoots were rooted and established in the field. Variations were observed in fruit traits like degree of russeting, fruit shape and size. The frequencies of the observed variations in fruit traits depended on the cultivar, ranging from 0.81 per cent in Doyenne d'Hiver to 3.64 per cent in Passe Crassane.

7. Choice of explants

Bhagwat and Duncan (1998) exposed two types of explants (Type I - Dissected apices and Type II Corms of *in vitro* derived shoots) of banana cv. Highgate to gamma irradiation. Radiosensitivity was assessed by the number of explants that survived. Eight weeks after irradiation, the percentage survival of Type I explants ranged from 100 with the untreated control to 38.20 at 5.00 kR. Generally, the survival was quite high (over 75 %) for the treatments 1.00 to 4.00 kR. In the case of Type II explants, explant survival ranged from 100 per cent with the control to 64.5 per cent for 4.00 kR treatments. With 5.0 kR treatment, only 29.40 per cent of the explants survived.

8. Mutation frequency

In *Solanum surattense*, Malpathak and David (1994) obtained enhanced levels of solasodine content through the irradiation of cell cultures at 60 Gy gamma rays, even though the growth in terms of cell number and dry weight was reduced. The effectiveness of X ray irradiation on regeneration of adventitious buds on *in vitro* leaf explants of three *Rosa hybrida* L. genotypes was studied by Ibrahim *et al.* (1998). X-ray doses of 0.5 and 1.5 kR were selected and variations were observed between the genotypes. Among the genotypes, clone RUI 317 had the highest rate of adventitious bud regeneration.

9. Mutagen effects on morphological changes

Mutations caused by physical mutagens resulted in the manifestation of cytological or biochemical effects. In Grapes, Charbaji and Nabulsi (1999) observed a notable increase in number of leaves, shoot length, root length and number of roots upon gamma irradiation (5 to 7 Gy).

The *in vitro* derived mutants of chrysanthemum produced highest percentage of chlorophyll variegation (55 %) and highest percentage of chlorophyll variegated leaves per mutated plants (5 %). Similarly the highest percentage of flower colour mutant (54 %) was observed with 2.5 kR gamma ray treatment (Mandal *et al.*, 2000 a). Later, Mandal *et al.* (2000 b) stated that somatic mutations in chrysanthemum flower colour were detected after irradiation with 1.5 and 2.0 kR gamma ray. The original colour of cv. 'Purnima' was white, while the mutated sector exhibited yellow colour. Similarly the original colour of cv. 'Colchi Bahar' was red and the mutated sector expressed yellow colour. In Banana,

From a gamma irradiated embryogenic callus of sweet potato, Lee *et al.* (2002) isolated morphological mutants with variation in chlorophyll deficient, purple stem, leaf type, shortened, thicker internodes and petioles. Similarly, in cultivar 'Yulmi', the mutation frequency of variants at 50 Gy dose was higher than that of other doses. But in the cultivar 'White star' the frequency of variation of the 90 Gy dose was the highest of all the doses (0, 30, 50, 70 and 90 Gy). Frequencies of the variants derived from irradiated callus ranged from 3 to 7.8 per cent, compared to 0.1 to 1.1 per cent in untreated control. Shobhana and Rajeevan (2003) exposed protocorms of *Dendrobium* hybrid Sonia x Emma White to various doses of gamma irradiation. The plantlets from the irradiated protocorms were found to be dwarfer than the normal ones. The leaves of some of the plantlets were broader and thicker.

In vitro cultures of chrysanthemum ray florets were irradiated with 0.5 Gy and 1 Gy gamma irradiation. Two mutants were obtained in the gamma irradiated plants (0.5 Gy). Both the mutants

were yellow coloured but one having flat spoon shaped ray florets similar to the original cultivar, while the other having tubular florets (Misra *et al.*, 2004). *In vitro* mutagenesis in chrysanthemum was investigated by Dao *et al.* (2006) and found that there is no variation was observed in control population. But the mutant populations treated with 1.0 and 3.0 kR gamma rays produced different variations including a range of abundant variation in number of petals and colour.

The biometrical characters of shoot tip derived *in vitro* mutants of coleus in exerted a gradual reduction with increase in dose of mutagen for the traits like plant height and number of laterals plant^{-1}. Similarly, the callus derived *in vitro* mutants produced tallest plants (51.00 and 63.85 cm) at 120 and 180 DAP respectively in 0.50 kR gamma rays + 175 mM EMS treatment. (Velmurugan, 2007).

10. Mutagen effects on cytological effects

In grapevine cv. Podarok Magaracha, gamma irradiation (95-100 Gy) of leaf explants increased tetraploid plant formation frequency of primary (7 %) and embryogenic callus (7.6 %) and some aneuploid plants were also found (Kuksova *et al.,* 1997). In *Coleus forskohlii,* Velmurugan (2007) and Velmurugan *et al.* (2008) observed that the untreated control callus culture derived *in vitro* plantlets registered 30 chromosomes. However the mutagenic treatment with 1.50 kR gamma rays + 200 M EMS showed 28 countable chromosomes consisting of lengthiest chromosome.

11. Application of RAPD markers in the screening of *in vitro* derived mutants

Khanuja *et al.* (1998) screened the somaclones of altered genotypes in *Mentha arvensis* using RAPD marker and isolated them at early stages of the growth. They used 12 primers and observed significant differences between the variants and the parent cv. Himalaya. Variant-1 was clearly distinguishable in the DNA profile particularly in case of primer MAP 04. Sweet potato cultivar 'Yulmi' regenerates from gamma treated callus showed variations of DNA levels with five primers out of ten primers used. However, the regenerates from non-irradiated callus did not show any variation. However the total polymorphism rate in cultivar Yulmi and White Star plants regenerated from irradiated calli were 29.9 and 28.6 per cent respectively (Lee *et al.*, 2002). Later, Kathiresan (2005) made an attempt to associate the polymorphism with forskolin content and tuber yield in the selected eight superior mutants and somaclones, using RAPD markers, with ten random primers. The results revealed that mutant did not express any polymorphism.

Velmurugan (2007) and Velmurugan *et al.* (2008) stated that the callus derived *in vitro* derived mutants with the mutagenic treatment of 1.50 kR gamma rays + 225 mM EMS produced significant polymorphic regions in coleus genome.

CONCLUSION

In vitro mutation technique is also become more and more important to prevent or restrict chimera formation Irradiation in combination with chemical mutagens has proved to be a valuable method in creating desirable variations followed by rapid propagation. As a result of following mutagenic treatments, a mixed bag of unexpected miracles of induced variations has been achieved in an array of horticultural crops. Realization during recent years in this field has enlightened the utility and effectiveness in horticulture crop improvement.

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