J Plant Biotechnol (2018) 45:55–62
DOI:https://doi.org/10.5010/JPB.2018.45.1.055

ISSN 1229-2818 (Print)
ISSN 2384-1397 (Online)

Research Article

Effect of gamma ray irradiation and ethyl methane sulphonate on *in vitro* mutagenesis of *Citrullus colocynthis* (L.) Schrad

D. Ramakrishna · G. Chaitanya · V. Suvarchala · T. Shasthree

Received: 13 February 2018 / Revised: 12 March 2018 / Accepted: 12 March 2018
ⓒ Korean Society for Plant Biotechnology

Abstract In the present study in vitro mutagenesis was used to study the effect of gamma irradiation and EMS on callus induction, morphogenesis and production of multiple shoots from different explants of *Citrullus colocynthis* (L.) Schrad. Gamma radiations (5 kR to 20 kR) and certain chemicals have been effected on plant growth developments and changes of biochemical metabolisms in plants. Murashige and Skoog (MS) medium containing with auxins such as NAA, IAA, 2,4-D (0.5 ~ 2.0 mg/l), cytokinines BAP, kn TDZ, (0.5 ~ 2.5 mg/l), L-Glutamic acid (1 ~ 2 mg/l) and Coconut milk (10 ~ 20%). After 5 weeks on induction media, explants and callus (EC) were exposed to 5 kR, 10 kR, 15 kR and 20kR, of gamma radiation and treated with 1, 2, 3, 4 and 5 mM ethyl methane sulphonate (EMS) for 30 min. The highest percentage of callusing was observed (70%) stem irradiated with 5 kR and significantly decrease in fresh and dry weight of callus in the below 4 kR doses and above 20 kR doses, there was a progressive decrease in the fresh weight and dry weights when compared to control callus. Maximum percentage of plantlet regeneration (59%) was induced from callus exposed to 15 kR gamma irradiation on MS media fortified with 2.0 mg/l 2,4-D + 2.0 mg/l BAP + 2.0 mg/l L-glutamic acid. Increase in gamma irradiation dose above 15 kR and 5 mM EMS reduced regeneration capacity of callus. Doses higher than 20 kR and 7 mM EMS was lethal to micropropagated plants of Citurullus colocynthis.

Keywords Mutagenesis, Morphogenesis, Gamma irradiation, Ethyl methane sulphonate, Citrullus colocynthis, Callus, Regenaration, Plantlets, Auxins, Cytokinins

D. Ramakrishna · G. Chaitanya · V. Suvarchala ·
T. Shasthree (✉)
Department of Biotechnology, Kakatiya University, Warangal – 506009, TS, India
e-mail: shastritaduri@gmail.com, tadurishasthree@outlook.com

Introduction

The combination of mutation breeding and "*in vitro* mutagenesis" is called mutagenesis. The source for most breeding material begins with mutations, whether the mutation occurs in modern cultivars, a landrace, a plant accession, a wild related species, or in an unrelated organism. It has been found to make induction and the selection of induced somatic mutations more effectively and regeneration of mutant plants. Mutations in a broad sense include all those heritable changes, which alter the phenotype of an individual. Genetic variation is the starting point of any breeding programme. Genetic variation may already be present in nature, may be obtained after several years of selection. Spontaneous somatic mutations have played an essential role in the speciation and domestication of plants. Unfortunately, the rate of occurrence of spontaneous mutation is too low to satisfy practical breeding needs. Plant breeding using the conventional procedure is time consuming and sometime for a number of plant species (Ehsanpour and Jones 2001). New technology such as tissue culture, gene transformation and mutation breeding technology are possible solutions to improve the productivity of modern agro ecosystem (D. Ramakrishna and T Shasthree 2016; Rudulier et al. 1984). Hence the combined use of mutation induction and *in vitro* technology is more efficient, because it speeds up the production of mutants as a result of an increased propagation rate and a greater number of generations per unit time and space (Espino 1986; Murpurgo 1997). The principle of *in vitro* mutagenesis is to device a scheme by which we can induce DNA lesions in a certain population of cells maintained *in vitro* and allow these cells to divide rapidly so that the repair mechanism introduces minor errors in the nucleotide sequence of the DNA. As a result, such cells have mutations in specific genes and if whole plants were regenerated from such cells, one would obtain mutant plant lines. The mutagens cause various kinds of DNA damage,

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

such as deletion or duplication of nucleotide segments of DNA in the chromosomes. Plant growth regulators and in particular a cytokinin-enriched medium can increase the recovery rate of mutated cells (T. Shasthree et al. 2012). Although the callus maintains its regeneration capacity for a longer period, prolonged subculturing may lead to higher frequency of mutants, especially in higher concentration of 2,4-D (Jureti and Jelaska 1991). Mutagenic agents such as radiations and certain chemicals can be used to induce mutations at a higher frequency and generate genetic variation from which desired mutants may be selected (Stover and Buddenhagen 1986; Van Harten 1998; De Langhe 1987). Proposed mutation techniques as tool for crop improvement. Several researchers suggested the use of mutagenic agent for induction of resistance to several diseases (Panton and Menendez 1972). Gamma irradiation is the main physical mutagen used to induce genetic variation. Novak (1986), Shasthree (2009) described the dose response of tissue-cultured shoot tips to gamma irradiation. Stotzky (1964) reported on the effect of gamma rays on seed germination and seedling survival in the diploid *Musa balbisiana.* However, a longer incubation period coupled with low concentration is preferred because it decreases damage causing hydrolytic by-products and thus improves the mutagenic efficiency (Walther 1969 and Savin 1968). Among physical mutagens frequently used on plant cell cultures are X-rays, UV-irradiation and γ-rays (CO^{60}) after determining the most suitable dosage and period of exposure. Ultraviolet light (UV) is convenient for mutagenesis. UV mutagenesis is important to incubate cells in dark so as to reduce photo-inducible DNA repair. Subjecting the minimum unit of explants to mutagen can be achieved through induction of mutation under *in vitro* condition of tissue culture and the opportunity for the mutated cells to survive get increased (Broerties et al., 1976). When callus exposed to UV-C the effect of drought stress on growth was lower than unexposed calluses. This could be due to genetic changes or epigenetic changes. The possibility of UV-C response in *Medicago* calluses for selection to osmatic stress using Poly Ethylene Glycol (PEG) added to MS medium according to diffusion based metho (Girma and Kreig 1992; Dami and Hughes 1997). The effect of UV-C doses on plant cells (e.g. protoplast and cell suspension) has already been tested in content of DNA mutagenesis in *Arabidopsis thaliana* with increasing UV-C doses, (Danon and Gallois, 1998). UV radiation can affect many aspects of plant process at the physiological and DNA level (Kolb et al. 2001; Woodall and Stewart 1998). Chemical mutagenesis is one of the major approaches for induction of mutation (Kharkwal et al. 2004). Chemical mutagen represents a powerful tool to enhance variability in plants for selection of new cultivars. Ethyl methane sulphonate (EMS) 50 mg/l most commonly used chemical. N-methyl-N-nitro-N-nitrosoguanine (NG), another mutagen, has successfully been used in deriving cell lines resistant to amino acid analogues. Heinz (1973) used methyl methane sulphonate (50 mg/l) and EMS to bring about mutations in sugarcane. EMS and 5-bromo-2-deoxyuridine have been used for broadening the range of alkaloid content of *Nicotiana sylvestris.* When plants are treated with EMS or a mutating chemical, lots of mutations arise in the DNA, but even these are difficult to detect with molecular marker methods (Bouman and De Klerk 2001).

There are very few reports on *in vitro* chemical mutagenesis. George and Rao (1980) studied the effect of EMS on cotyledon cultures of mustard plants. Gosh et al. (1984) reported the effect of EMS of legume tissue culture. Mustafa et al., (1991) reported induction of multiple shoots from cotyledon culture of cucumber (*Cucumis sativus)* by MMS. Most of the mutations recovered in tomato were identical with the already known spontaneous or induced mutations, but some mutations were entirely new e.g., jointless pedicel mutant in tomato which is highly valued for mechanical picking. Murugan and Subramanian (1993), observed variability in progenies in Cowpea varieties upon radiation with gamma rays and the analysis of variance between progeny and within progeny were highly significant (Lawhale 1982; Kharkwal 2001). In order to obtain tetraploid plants from diploid day-lily callus, colchicines has been used (Chen and Geoden-Kallemeyn 1979). For an efficient mutation breeding programme, the knowledge on efficiency of mutagens being used is of basic importance (Singh et al. 1997, Kumar and Narayan 2005). Mutation frequency, mutagenic effectiveness and efficiency were calculated on M_2 generation as per (Konzak et al., 1965). Mahana et al., (1990) observed several chlorophyll and morphological mutants in Cowpea (*Cicer arientium)* upon treatment with EMS. Relatively higher mutation frequency both for chlorophyll and morphological mutations were observed for EMS and MMS.

There were no reports of *in vitro* mutagenic studies in *Citrullus colocynthis* L. In the present investigation the effect of gamma rays on morphogenesis rooting efficiency, caulogenesis and number of shoot production were studied. Several crop plants were subjected to mutation *in vitro* (both physical and chemical) for desirable characters such as yield, quality, resistance, tolerance (Evolva et al., 1983). These were considerable work on *in vitro* mutagenesis especially followed gamma-irradiation on seeds, explants

callus culture and seedling for their morphological and physiological variations.

Materials and Methods

Citrullus colocynthis plants were used in the present investigation and seeds were collected from river valleys of Koonoor Warangal, Andhra Pradesh, Basara Nizamabad Telangana State and Bharathidasan University, Thiruchunapally, Tamilnadu, India. The collected plants were maintained in the department green house at Kakatiya University. Leaf, cotyledon, stem and node explants were collected from the green house grown plants and washed thoroughly in running tap water for 5 to 10 min under aseptic conditions. Explants were surface-sterilized by dipping in 0.1% fresh aqueous mercuric chloride (HgCl2) solution for 1 to 2 min and subsequently washed thoroughly with double-distilled water to remove traces of HgCl2. The pH of the medium was adjusted to 5.6 to 5.8 by using either 0.1N NaoH or 0.1N HCl before autoclaving. About 10 ml of the medium was dispensed in each culture tube and sealed with nonabsorbent cotton plugs prior to autoclaving at 121°C for 15 min under 15 psi. Sterilized cotyledon and leaf explants were cultured on MS sterilized medium supplemented with various concentrations of auxins and cytokinine and incubated at 25 ± 1°C under a 16/8-h (light/dark) photoperiod provided by cool white fluorescent tubes (Crompton India Ltd.) with light intensity of 2,000 lux. After 5 weeks on induction media, explants and callus (EC) were exposed to 5 kR, 10 kR, 15 kR and 20 kR, of gamma radiation (GR) in a gamma cell (60Co source) installed at the instrumentation department, Kakatiya University. After irradiation, EC was transferred to fresh medium. For ethyl methane sulphonate (EMS) treatments, the appropriate amounts of EMS was mixed separately in MS basal medium and dissolved thoroughly for each treatment. The pH of the medium was adjusted to 6.0 EMS, respectively, prior to adding the mutagens. The solutions were filter-sterilized with sterile Millipore 0.45 μm membrane filter in the laminar air flow chamber. The EC was treated with 1, 2, 3, 4 and 5 mM EMS for 30 min. The doses/concentrations of both physical and chemical mutagens were selected. The treated EC were thoroughly rinsed with sterile MS liquid medium (basal) to rinse out any excess mutagens and blotted dry on sterile tissue paper. Then untreated EC (controls) and treated EC were cultured on above mentioned induction medium for 2 ~ 3 weeks to assess the survival rate of ECs and then plated on maturation medium containing MS salts, 3% (m/v) sucrose and different levels of plant growth regulators (PGRs) such as 0.5 to 2.5 mg/l BAP, 0.5 to 2.0 mg/l 2,4-D, 1.0 to 1.5 mg/l IAA, 1.0 to 2.0 mg/l NAA, 0.5 to 2.0 mg/l TDZ, 1.0 to 2.0 L-glutamic acid, 1.0 to 1.5 kn, 10 to 20% Coconut milk (CM) and 0.7% agar. The observations were made the callus texture, color, shoots and root ratio from treated explants and callus of *C. colocynthis*. Morphological variations of the regenerated plants during the developmental stages were noted with reference to the control. Cultures were incubated for 10 to 15 d and plantlets with well developed roots were transferred to plastic cups containing autoclaved mixture of sand, red soil, and vermicompost (1:1:1). Each plantlet was covered with a polythene bag and the cups were maintained in controlled temperature and 60% relative humidity. After hardening, well established plants (R_0 plants) were transferred to the pots (40 × 45 cm), and grown to maturity under recommended agricultural practices. R_0 plants produced normal flowers and viable seeds in pods. Each treatment consisted of at least 20 explants and the experiment was repeated thrice. The data was analysised using ANOVA.

Results

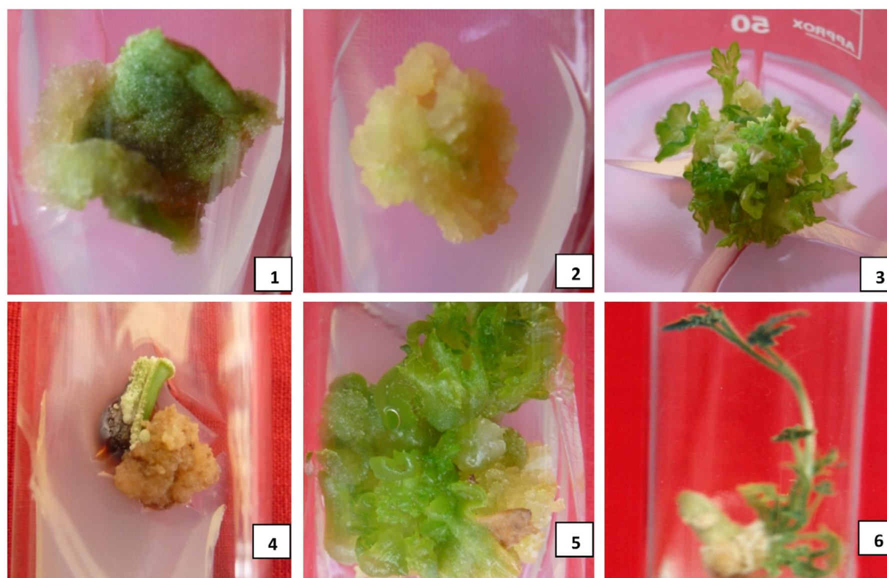
Effect of Gamma rays

Gamma rays irradiated explants

In the present investigation, the effect of gamma rays on induction of callus and organogenesis was studied from different explants i.e., leaf, cotyledon, stem and nodal. Attempts have been made to determine the most effective dose of physical mutagen which could induce a maximum number of shoots per explant and the dose that inhibit the shoot bud initiation were also ascertained. Induction of shoots in meristematic tissue and seedling explants of *Citrullus colocynthis* (L.) Schrad were under investigation after treating with gamma irradiation.

Cotyledon explant culture

Cotyledon explants of *Citrullus colocynthis* (L.) Schrad were irradiated to 5 kR gamma irradiations and were inoculated on MS medium with 1.5 mg/l 2,4-D and 1.5 mg/l BAP. Proliferation of callus from cut ends was showed (Fig. 1). After six weeks of subculture on the above same medium callus has been turned to dark brown (Fig. 2). The cotyledon derived callus was irradiated to 10 kR, started proliferation after 15 days of inoculation. In 65% of cultures show the callus proliferation on 2.0 mg/l 2,4-D



[1] Effect of different doses of gamma irradiations (5 KR-20 KR) on different explants of *Citrullus colocynthis* (L.) Schrad

Fig. 1 5 KR irradiated, cotyledon explants on MS medium with 1.5 mg/l 2,4-D and 1.5 mg/l BAP

Fig. 2 5 KR irradiated, six weeks cotyledon callus subcultured on MS medium with 1.5 mg/l 2,4,D and 1.5 mg/l BAP

Fig. 3 Few shoots induced on the same medium, addition of 1.0 mg/l TDZ + 15% CM

Fig. 4 5 KR ,10KR and 15KR dose irradiated on stem explants cultured on MS medium with 2.0 mg/l 2,4-D + 1.0 mg/l BAP+ 1.0 mg/l L-glutamic acid

Fig. 5 5 KR irradiated callus turned into green colour after eight weeks on the same medium

Fig. 6 15 KR irradiated callus produced plantlets on MS medium with 2.0 mg/l 2,4-D+ 2.0mg/l BAP+ 2.0mg/l L-glutamic acid

Table 1 Effect of 2, 4-D, BAP, TDZ and cm on differentiation from Cotyledon derived callus of *Citrullus colocynthis* (L.) exposed to gamma irradiation (5 kR)

Harmone (mg/l)	Leaf derived Callus	
	% frequency of growth response	Morphogenetic response
1.0 2, 4 - D + 1.5 BAP	58	Excessive White Callus
1.5 2, 4 - D + 1.5 BAP	65	Compact brown callus
2.0 2, 4 - D + 1.5 BAP	60	Friable Callus
1.5 2, 4 - D + 2.0 BAP	52	Compact Callus
2.0 2, 4 - D + 2.0 BAP	68	Callus with roots
2.5 2, 4 - D + 2.0 BAP	42	Hard Callus
1.5 BAP + 1.0 TDZ +15% CM	48	Greening of Callus
2.0 BAP + 1.0 TDZ +15% CM	52	Callus with shoot buds
2.5 BAP + 1.0 TDZ + 15%CM	50	Plantlets formation

Data scored at the end of five weeks, 50 days of 2 callus from 10 replicate cultures

and 2.0 mg/l BAP. The addition of 1.0 mg/l TDZ + 2.0 mg/l BAP + 15% CM to the media few shoots were induced from 10 kR irradiated long term callus (Fig. 3).

There was a significant increase in fresh and dry weight of callus as well as morphogenetic response with low doses where as at higher doses there was progressive decrease in the fresh and dry weights (Table 3).

Stem explant culture

In order to increase the frequency of shoots, the stem explants were irradiated from 5 kR dose to 15 kR dose on MS medium with 2.0 mg/l 2,4-D + 1.0 mg/l BAP + 1.0 mg/l L-glutamic acid (Fig. 4). The frequency of growth response and morphogenetic response was recorded (Table

Table 2 Effect of 2, 4-D, BAPand L-glutamic acid on differentiation from stem derived callus of *Citrullus colocynthis* (L.) Schrad callus exposed to gamma irradiation (5 KR)

Harmone (mg/l)	Leaf derived callus	
	% frequency of growth response	Morphogenetic response
0.5 2, 4 − D + 1.0 BAP + 0.5 L- glutamic acid	62	Callus formation
1.0 2, 4 − D + 1.0 BAP + 1.0 L- glutamic acid	68	White friable callus
2.0 2, 4 − D + 1.0 BAP + 1.0 L- glutamic acid	70	Excessive callus
2.5 2, 4 − D + 1.5 BAP + 1.0 L- glutamic acid	55	Compact callus
1.0 BAP + 1.5 L- glutamic acid	45	Greening of callus
1.5 BAP +2.0 L- glutamic acid	58	Globular green callus
2.0 BAP + 2.5 L- glutamic acid	42	Browning of callus
0.5 2, 4 − D + 2.0 BAP + 1.0 L- glutamic acid	50	Green spots on callus
1.0 2, 4 − D + 2.0 BAP + 1.5 L- glutamic acid	52	Few shoot buds
2.0 2, 4 − D + 2.0 BAP + 2.0 L- glutamic acid	59	Plantlet regeneration
2.5 2, 4 − D + 2.0 BAP + 2.5 L- glutamic acid	40	NR

Data scored at the end of five weeks of culture.; NR − No Response

Table 3 Morphogenetic response of leaf explants derived from gamma irradiated seedlings on MS medium with TDZ, NAA and BAP in *Citrullus colocynthis* (L.)

dose (kr)	% of culture with growth response	morphogenetic response
Control	62	Callus
1	65	excessive callus
2	70	white friable callus
3	74	callus with 1-2 roots
4	80	greening of callus
5	85	callus with shoot buds
10	90	plant regeneration
15	55	compact callus
20	Nr	Nr
25	Nr	Nr

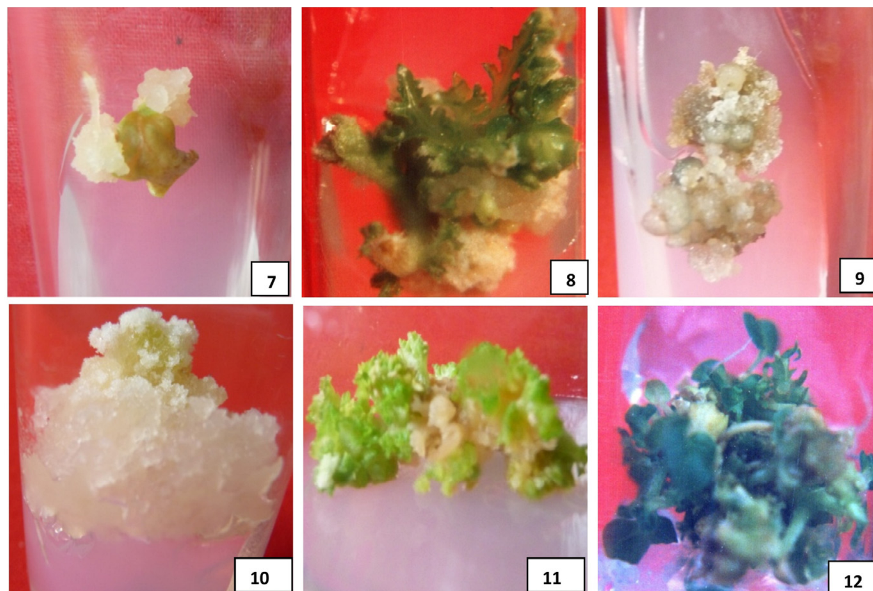
Date scored at the end of 5 weeks of culture; nr − no response

2). The highest percentage of callusing was observed (70%) stem irradiated with 5 kR. After eight weeks of subculture on MS medium with 1.5 mg/l BAP and 2.0 mg/l L-glutamic acid, greening of callus was induced (Fig. 5). Maximum percentage of plantlet regeneration (59%) was induced from callus exposed to 15 kR gamma irradiation on MS media fortified with 2.0 mg/l 2,4-D + 2.0 mg/l BAP + 2.0 mg/l L-glutamic acid (Fig. 6).

Leaf explant cultures

Young leaves from *in vitro* germinated seedlings were exposed to varying doses e.t., from 1 kR to 20 kR. 5 kR irradiated leaf explant was inoculated on MS medium with 1.5 mg/l TDZ and 1.5 mg/l NAA (Fig. 7) started proliferation after 15 days of inoculation. In 80% of cultures show the callus proliferation on MS medium with 2.0 mg/l TDZ and 1.5 mg/l BAP. After callus exposed to 20 kR doses on MS medium with 2.0 mg/l TDZ and 2.0 mg/l NAA shoot buds were induced. Addition of 3.0 mg/l TDZ and 2.0 mg/l BAP to the media plantlets were regenerated after callus exposed to 20 kR gamma irradiation (Fig. 8).

There was a significant increase in fresh and dry weight of callus in the low doses, whereas at higher doses, there was a progressive decrease in the fresh weight and dry weights when compared to control callus. Regeneration of plantlets induced from the callus exposed to 10 kR gamma irradiation (Table 3).



[2] Effect of different doses of gamma irradiations (5 KR–20 KR) on different explants of *Citrullus colocynthis* (L.) Schrad

Fig. 7 5KR irradiated leaf on MS medium with 1.5 mg/l TDZ and 1.5mg/l NAA

Fig. 8 2OKR irradiated leaf callus produced regenerated plantlets on MS medium with 3.0mg/l TDZ and 2.0 mg/l BAP

Fig. 9 Nodal segment treated with 0.25% ems and inoculated on MS media fortified with 1.5mg/l 2,4-D and 1.0mg/l IAA

Fig. 10 White friable callus induced on MS media with 1.5mg/l 2,4-D and 1.0mg/l IAA

Fig. 11 Green callus with multiple shoots formed on MS media with1.5mg/l 2,4-D and 1.0mg/l IAA

Fig. 12 Multiple shoots were initiated when treated with 0.25% EMS and cultured on MS media with2.0 mg/l KN and 1.0 mg/l L-glutamic acid

Table 4 Morphogenetic response of nodal explant derived from ems (0.25%) treated seedlings of *Citrullus colocynthis* (L.) schrad on ms with 2,4-D, IAA, KN and L – glutamic acid

Treatment (h)	Node	
	% frequency of growth response	Morphogenetic response
Control	65	Callus
0.1% EMS		
6	54	Initiation of Callus
12	45	White Friable Callus
18	40	Brown Callus
24	32	Dark Brown Callus
0.25% EMS		
6	45	Greening of Callus
12	50	Multiple Shoot Inductia
18	18	Browning of Callus
24	NR	NR

Data recorded at after nine weeks of cultures; NR- No response

Nodal explant culture

In the present study to increase the frequency of shoots, the nodal explants were treated with 0.25% EMS and inoculated on MS media fortified with 1.5 mg/l 2,4-D and 1.0 mg/l IAA (Fig. 9). White friable callus was induced on the same medium after subcultures (Fig. 10). Greening of callus induced few shoots on the same medium with 2.0 mg/l 2,4-D and 1.5 mg/l IAA (Fig. 11). Multiple shoots were initiated when treated with 0.25% EMS and cultured

on MS media with 2.0 mg/l Kn and 1.0 mg/l L-glutamic acid (Fig. 12). Amino acids play an important role in induction of shoots.

Discussion

In general, mutagenic treatments are not applied to cell cultures for the recovery of somaclonal variants. But in those studies where mutagenic treatments were used, usually an increase in the frequency of somaclonal variants was observed. In some cases, mutagenesis was reportedly necessary for the recovery of the specific variant being isolated. Gamma irradiation is the main physical mutagen used to induce genetic variation. The combined use of mutation induction and *in vitro* technology is more efficient solution to improve the productivity of modern agro ecosystem (Rudulier et al. 1984). Genetic variation is the starting point of any breeding programme. A combination of explants irradiation and *in vitro* regeneration is mostly effective for manifestation of variants. Novak (1987) described the dose response of tissue cultured shoot tips to gamma irradiation. In the present study *in vitro* mutagenesis was used to study the effect of gamma irradiation and EMS on callus induction, morphogenesis and production of multiple shoots. The effect of gamma rays in tissue culture has been reported in different explant material (Shasthree et al. 2009; Degani and Pickholz 1973). In the present study, lower doses of irradiation favoured callus growth than higher doses. During the study, various variations were observed in leaf, floral characters, callus induction and shoot formation. These findings were supported by (Shasthree et al. (2009). Mustafa et al. (1993) reported effect of gamma irradiation on morphogenesis from different explants of *Momordica charantia.* Bajaj (1970) used different doses of 0, 1, 2, 3, 10 & 20 kR gamma rays on callus cultures of *Phaseolus vulgaris* to study their effect on total protein and RNA. The response of cells to radiations are said to be dose dependent according to (Arya and Hilbrandt 1969) working on grape stem callus. There were few reports on *in vitro* chemical mutagenesis. George and Rao (1980) studied the effect of EMS on cotyledon cultures of mustard plants. The effect of gamma irradiation on growth and cytology of carrot tissue culture was reported by (Bassam Alsafadi and Simon 1990).

Conclusion

Optimal level mutagenic agents such as gamma radiation and certain chemicals, like ethyl methane sulphonate (EMS) have been playing an important role in the crop growth, development and enhancement of secondary metabolites in plants. Moreover, lower levels of gamma irradiation and ethyl methane sulphonate (EMS) are reduced regeneration capacity of callus and the higher dose of gamma radiation and EMS was lethal to micropropagated plants of *Citurullus colocynthis*. So this paper could be helpful for understanding the effect of gamma irradiation and ethyl methane sulphonate in plant system.

Acknowledgments

The Principal Investigator Dr. T. Shasthree is thankful to UGC New Delhi for financial assistance in the form of Major Research Project Vide F. No.: 41-530/2012 (SR) during July 2012- July 2015 for this work.

References

Arya HC, Hilbrandt AC (1969) Effect of gamma irradiation on callus growth of *Phyllonera* gall and normal grape stem cells in tissue. Can J Bot 47:1623-1628

Bajaj YPS (1970) Effect of gamma radiation on growth, RNA protein and nitrogen contents of bean callus cultures. Ann Bot 34:1084-1096

Bassam AL, Safadi, Phillip W Simon (1990) The effect of gamma irradiation on the growth of cytology of carrot (*Daucus carota* L.) tissue culture. Envr and Exptl Bot 30(3):361-371

Bauman H, De Klerk GJ (2001) Theor. Appl Genet 102:111-117

Broerties C, Van Hasten AM (1976) Ornamental crops. Development in crop science (2): Application of Mutation Breeding Methods in the Improvement of vegetatively Propagated Crops. Elsevier Scientific Publishing Company, Amsterdam, pp 76

Chen CH, Goeden-Kallemeyn YC (1979) *In vitro* induction of tetraploid plants from colchicines-treated diploid daylily callus. Euphytica 28:705-509

D. Ramakrishna, T. Shasthree (2016) High efficient somatic embryogenesis development from leaf cultures of Citrullus colocynthis (L.) Schrad... Article in Physiology and Molecular Biology of Plants · June 2016

Dami I, Hughes HG (1997) Effecdt of PEG-induced water stress on *in vitro* hardening of Valiant grape. Plant Cell Tiss Org Cult 47:97-101

Danon A, Gallois P (1998) UV-C radiation in duces apoptotic-like changes in Arabidopsis thaliana. FFBs Lett 437:1310136

De Langhe E (1987). Towards an international strategy for genetic improvement in the genus *Musa,* Banana and Plantain Breeding Strategies, ACIAR, Canberra 21:19-23

Degani N, Pickholz D (1973) Direct and indirect effect of gamma

irradiation on differentiation of tobacco tissue culture. Rad Bot 15:363-366

Ehsanpour AA, Jones MGK (2001) Plant regeneration from mesophyll protoplasts of potato (*Solanum tuberosome* L.) cultivar Delaware using silver thiosulfate (STS). J Sci 12:103-110

Espino RRC (1986) Mutation breeding on selected Philippine fruit crops, Nuclear Techniques and In vitro culture for Plant Improvement, IAEA, Vienna, 429-433

Evolva, S.V. (1983). The use of genetic markers selected *in vitro* for the isolation and genetic verification of interspecific somatic hybrids of *Nicotiana tobacum.* Mol. Gen. Genet., 189-141

George L, Rao PS (1980) *In vitro* regeneration of mustard plants (*Brassica juncea* var Rai-5) on cotyledon explants from non-irradiated, irradiated and mutagen treated seeds. Ann Bot 46:107-112

Girma FS, DR Kreig (1992) Osmatic adjustment in *Sorghum*. Plant Physiol 99:577-582

Gosh Mitra GC, Sharma AK (1984) Indian Bot Reptr. Prof. K.B. Deshpande. Commemoratin Vol. 70

Heinz DJ (1973) Sugarcane improvement through induced mutations using vegetative propagules and cell culture techniques. In: Induced mutation in vegetatively propagated plants, International Automic Energy Agency, Vicnna. p 53

Jureti B, Jelaska S (1991) Plant development in long-term embryogenic callus lines of *Cucurbita pepo.* Plant Cell Rep 9:623-626

Kharkwal MC (2001) Induced mutations in chickpea (*Cicer arientinum* L.) V. Evaluation of micro-mutatins. India J. Genet. 61:115-124

Kharkwal MC, Pandey rN, Pawar SE (2004) Mutation Breeding for Crop Improvement. 601-646. In: "Plant Breeding-Mendelian to Molecular Approaches", H.K. Jain and M.C. Kharkwal (eds.), Navosa Publishing House (P) Ltd., New Delhi.

Kolb CA, Kaur MA, Kopecky J, Zota G, Riedeve M, Pfundel EF (2001) Effect of natural intensities of visible and ultraviolet radiation on epidermal UV screening and photosynthesis in grape leaves. Plant Physiol 127:863-875

Konzak CP, Nilan RA, Wagnor J, Foster RJ (1965) Efficient chemical mutagenesis. Radiat. Bot. (suppl.) 5:49-70

Kumar D, Narayan P (2005) Production Technology of cowpea, AICRP on and Legumes, CAZRI, Jodhpur, Rajasthan, India

Lawhale AD (1982) Note on the genetic variability in quantitative characters of cowpea in M_3 generation. Indian J Agric Sci 52:22-23

Mahana SK, Bhargava A, Mohan L (1990) Alkaline azide mutagenecity in cowpea. Mut Breed News 36:6-7

Murpurgo R (1997) Enigma of banana breeding. A challenge for biotechnology Agro-Food Industry Hi-Tech July/August, 16-21

Murugan S, Subramanian M (1993) Variability studies for polygenic traits in M_3 & M_4 generations of cowpea (*Vigna aunguiculata*). Crop Res Hissar 6:264-269

Mustafa MD, Mallaiah B (1991). *In vitro* adventitious shoot formation from stem segments of snake gourd (*Trichosanthes anguina* L.). J Mendal 8(3):147-148

Mustafa, MD. Parthasarathy, M. and Mallaiah, B. (1991). Methyl Methane Sulphonate (MMS) induced multiple shoots from cotyledon cultures of cucumber (*Cucumis sativus* L. var Poinsett) Ad Plant Sci 4(2):419-422

Novak FJ (1986) Mutation induction by gamma irradiation of *in vitro* cultured shoot-tips of banana and plantain. Trop Agr (Trinidad) 67(1):21-28

Novak FJ, Micke A (1987) *In vitro* mutants technology for crop improvement in developing countries, Sabrao 19:82-85

Panton CA, Menendez T (1972) Possibilities and implication of mutatin breeding in Jamaica, IAEA, Vienna, 61-65

Rudulier DL, Strom AR, Dandekar AM (1984) Molecular biology of osmoregulation. Science 224:1064-1068

Savin VN (1968) Enhancement of chemically induced mutation frequency in barley through alteration in the duration of presoaking of seeds. Mut Res 6:101-107

Shasthree T, Chandrashekar Ch, Savitha R, Imran (2012) Effect of various plant growth regulators on callus induction from different explants of *Citrullus colocynthis* (L.) schrad. International Journal of Universal Pharmacy and Life Sciences 2(3)

Shasthree T, Imran MA, Narsinga Rao N, Mallaiah B (2009) Effectof physical mutagen (Gamma rays) on differentiation and multiple shoot production from different explants of *Erithrina variegata* L. BTAIJ 3(4):261-266

Singh BB, Chambhss OL, Sharma B (1997) Recent advances in cowpea breeding. In: B.B Singh, Mohan Raj, K. Dashiel, Jackai, L.E.M. (eds.). Advances in cowpea research. UTA/ JIRCAS, ITTA, Ibadan, Nigeria pp 30-49

Stotzky G (1964) Some effects of gamma irradiatin on seeds and rhizomes of Musa. Am J Bot 51:724-729

Stover RH, Buddenhagen IW (1986) Banana breeding: polyploidy, disease reisistance and productivity, fruits 40:175-191

Van Harten AM (1998) Mutation Breeding: Theory and Practical Applications, Cambridge University Press, Cambridge

Walther F (1969) "Effectiveness of mutagenic treatments with ionizing radiatins in barley. In: Induced Mutatins in Plants", Proceedings of a FAO/IAEA Symposium on the Nature, Induction and Utilization of Mutations in Plants, Pullman, WA, 261-270

Woodall GS, Stewart GR(1998) Do anthocanin play a role in UV protection of the red juvenile leaves of Syzygium. J Exp Bot 49:1447-1450