

In-vitro Mutagenesis in an Endangered Medicinal Cucurbit *Bryonopsis laciniosa* (L.) Naud

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Abstract

Bryonopsis laciniosa (L.) Naud. is an endangered and highly valuable medicinal cucurbit. The bioactive molecules tri - terpene glycosides, saponin and goniotalamin were isolated from the whole plant. The objective of the present study was to determine the most effective dose of physical mutagen and also to find the effect of chemical mutagen on different explants i.e. leaf, stem, nodal and cotyledon. Explants were irradiated with gamma rays and treated with EMS separately and were inoculated on MS medium supplemented with BAP, Kn 2, 4 – D, TDZ, NAA and L – glutamic acid. Nodal explant culture showed high potent plant regeneration after the callus exposed to 10 kR gamma irradiation. 0.25 EMS was found as the most suitable concentration for direct shoot induction.

Keywords: *Bryonopsis laciniosa*, goniotalamin, *in – vitro* mutagenesis, gamma rays, Ethyl methane sulphonate.

Introduction

Bryonopsis laciniosa (L.) Naud is an endangered and highly valuable medicinal plant belongs to the family Cucurbitaceae. Ethnobotanically the whole plant is attributed with pharmacological properties. The bioactive molecules triterpene-glycosides, saponin and goniotalamin were isolated from the whole plant. *Bryonopsis* is used to treat various types of diseases like asthma, bronchitis, colic, cholera, paralysis, tuberculosis etc. Mutagenesis *in – vitro* is an important field for crop improvement (King, 1984). Induced mutations are of great use for plant breeding, either directly to improve specific traits or indirectly for cross breeding experiments Negruțiu (1990). Plant culture methods are now finding applications of variants and varieties resistant

to various mutagens. Induced genetic variability in plant cells by physical and chemical mutagens may help to isolate mutants. Application of physical and chemical mutagens in tissue culture has been reported (Bottino, 1975). A combination of explant irradiation and *in – vitro* regeneration is mostly effective for manifestation of variants (Novak and Micke, 1987). Considerable options led to practicability of the mutagenesis techniques for improving productivity of a vast array of organisms useful to man (Chopra and Sharma, 1985). The application of *in – vitro* mutagenesis has vast potential for increasing the available genetic variants in the years to come. So far 2,200 mutant varieties of plants had been released world wide including 175 crop plant species with induced mutant varieties (Maluszynski *et al.* , 2000).

Ethyl methane sulphonate (EMS) is most commonly used chemical mutagen in *in – vitro* mutagenesis. A few traits were selected after treatment with this mutagen (Flick *et al* 1983). N – Methyl – N – nitro - N – nitroguanine (NG), another mutagen has successfully been used in deriving cell lines resistant to aminoacid analogues (Colijn *et al.*, 1979). Efficiency of its use upon proper optimization of the mutagenic treatment, production and characterization of albino mutants using Nitrosomethyl urea (NMU) as a mutagen applied to tomato seeds, have been extremely efficient and can also be used *in – vitro*. Chemical mutagens represent a powerful tool to enhance variability in plants for selection of new cultivars. Heinz (1973) used Methyl methane sulphonate and Ethyl methane sulphonate to bring about mutation in sugarcane. EMS and 5 – Bromo – 2 – deoxyuridine have been used for broadening the range of alkaloid content of *Nicotiana sylvestris*. Colchicine has been used to obtain tetraploid plants from diploid day lily (Chen and Goeden –Kallemeyn, 1979).

The physical mutagens in plant tissue culture has been reported by several authors (Devreux,1973;Street,1974;Skirvin,1978). There has been numerous investigations on the effect of ionizing radiation on callus tissue. Verma and Huystee(1971) observed the formation of giant cell in ground cell suspension cultures because of ionizing radiation(500 kR of gamma rays). Stimulatory effect of low doses of ionizing radiation not only on growth but also on different cultured plant cells; was demonstrated by several workers (Sharma *et al.*, 1983; Jain *et al.*, 1984, Degani ,1975.). Kochba and Spiegel Roy (1978) demonstrated that by irradiation and addition of certain growth regulators like IAA to the medium the response of *Citrus sinensis* tissue culture was enhanced. Among the physical mutagens frequently used on plant cell cultures are x – rays, UV – irradiation and Y – rays (⁶⁰Co) after determining the most suitable dosages and period of exposure. Several crop plants were subjected to mutation *in – vitro* (both physical and chemical) for desirable characters such as yield, quality, resistance and tolerance (Maliga *et al.*, 1982; Lazar *et al.*, 1983; Evolva *et al.*, 1983).Radiations are potentially useful for introducing plant mutants through tissue culture.

There are very few reports *in vitro* chemical mutagenesis. Ghosh *et al.*, (1984) reported the effect of EMS of legume tissue culture. Mustafa *et al.*, (1991) reported induction of multiple shoots from cotyledon cultures of cucumber (*Cucumis sativus*) by MMS (Methyl methane sulphonate). So far there are no reports on *in – vitro* mutagenesis in *Bryonopsis laciniosa*.

The main objective of the present study was to determine the most effective dose

of the physical mutagen and chemical mutagen on different explants like stem, leaf, cotyledon and nodal.

In the present study the effect of gamma irradiation (physical mutagen) and Ethyl methane sulphonate (EMS) a potential alkylating agent (chemical mutagen) on morphogenesis were studied. Shoots and rooting efficiency and callusing ability from different explants were used to study the effect of mutagens.

Materials and Methods

Seeds and plants were collected from the local forest area of Warangal Dist. and developed in the University campus garden. Leaf, cotyledon, nodal and stem explants were excised from the university campus garden grown plants and washed thoroughly in running tap water for 10 min, then in 70% alcohol for 5 min and surface sterilized with 1.0 % aqueous Mercuric chloride solution for 5 min and rinsed with sterile distilled water for 3-4 times to discard the traces of mercuric chloride . The explants were cultured on MS medium fortified with different concentrations of plant growth regulators along with 3% sucrose (w/v) and 0.8% (w/v) of agar. The PH of the medium was adjusted to 5.7 to 5.8 using 0.1 N NaoH or 0.1 N HCl before autoclaving. About 10 ml of the medium were dispensed in each culture tube and sealed with non – absorbent cotton plugs prior to autoclaving. The culture tubes were autoclaved at 121⁰C for 20 minutes and maintained at 16 hr photoperiod with – 2000 lux light.

The explants used for *in vitro* mutagenetical experiments were gamma irradiated with dose of 1.0, 2.0, 3.0, 4.0, 5.0,10,15 and 20 kRs at central instrumentation center KU, and also the explants were treated with Ethyl methane sulphonate (EMS) an alkylating agent from 0.1 and 0.2% concentrations.

Results and discussions

In the present study, the effect of gamma rays on induction of callus and organogenesis were studied from various explants i.e. leaf, cotyledon, nodal and stems. Attempts have been made to determine the most effective dose of physical mutagen which could induce highest number of shoots per explants, and the dose that could inhibit the effect on shoot bud initiation were also ascertained. Induction of shoots in meristematic tissue and seedling explants of *Bryonopsis lacinoisa* were under investigation after treatment of gamma irradiation and also the effect of chemical mutagen on morphogenesis from different explants were observed.

Effect of physical mutagen

Nodal explant cultures

The nodal explants irradiated with 5 kR of gamma rays and were cultured on MS medium 2.0 mg/l BAP and 1.0 mg/l 2,4 – D and 1.0 mg/l NAA induced callus and produced 1 to 2 roots from nodal explants. (Plate I, Fig.1) A white hard callus was produced when the explants cultured on MS medium containing 1.0 mg/l 2, 4 – D and 2.0 mg/l BAP. (Plate I; Fig 2). The callus derived from the nodal explants were

exposed to 5 kR gamma irradiation and cultured on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l TDZ and 20% CM initiated regeneration. (Plate I, Fig.3). Addition of amino acid (2.0 mg/l L – glutamic acid) to the medium containing 2.0 mg/l BAP and 1.0 mg/l 2,4 – D a plant was regenerated after the callus exposed to 10 kR gamma irradiation. (Plate I, Fig.4).

At lower doses (1, 2, 3, and 4kR) there was no significant stimulation of growth in the callus. From 5 to 20 kR there was a gradual decrease in growth. At 10 and 15 kR there was no visual sign of growth for the first two weeks. At higher dose like 20 kR growth was drastically reduced. Among the explants tested nodal explant culture showed high potent plant regeneration with 10 kR gamma irradiation.

Plate I

Effect of Gamma rays on organogenesis from nodal explant cultures in *Bryonopsis laciniosa* (L.) Naud.

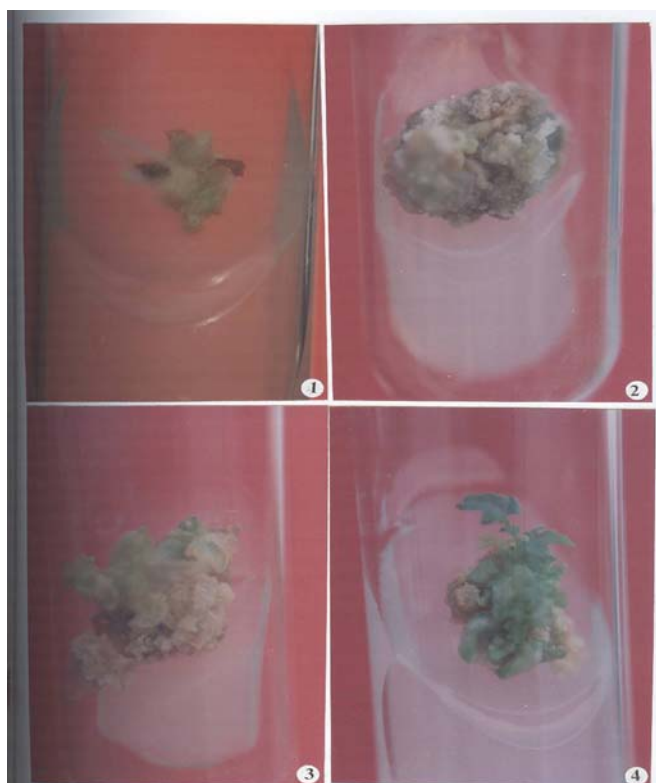


Figure 1: Induction of callus and 1 to 2 roots from nodal explant after exposed to 5 kR on MS + 2.0 mg/l BAP + 1.0 mg/l 2,4 – D + 1.0 mg/l NAA. **Figure 2:** White hard callus on MS + 1.0 mg/l 2,4 – D + 2.0 mg/l BAP. **Figure 3:** Initiation of regeneration from callus derived from nodal explants after exposed to 5 kR gamma irradiation on MS + 2.0 mg/l BAP + 1.0 mg/l TDZ + 20% CM. **Figure 4:** Plant regeneration after the callus exposed to 10 kR gamma irradiation on MS + 2.0 mg/l BAP + 1.0 mg/l 2,4 – D + 2.0 mg/l L – glutamic acid.

Effect of chemical mutagen (ETHYLMETHANE SULPHONATE)

Stem explant cultures

In order to increase the frequency of shoots, the stem explants were treated with 0.25 EMS and cultured on MS medium containing 1.0 mg/l 2,4 – D and 1.0 mg/l NAA produced dark brown callus (Plate II, Fig.1).

Callogenesis and direct shoots were induced when cultured on MS medium supplemented with 1.0 mg/l Kn and 1.0 mg/l L – glutamic acid (Plate II, Fig.2). Amino acids play an important role in induction of direct shoots.

Various explants like stem ,leaf, nodal and cotyledon were tested , among the explants tested stem explant culture induced callogenesis and regeneration when treated with 0.25 EMS.

Plate II

Effect of 0.25 EMS on callogenesis and regeneration from stem explant cultures of *Bryonopsis laciniosa* (L.) Naud.

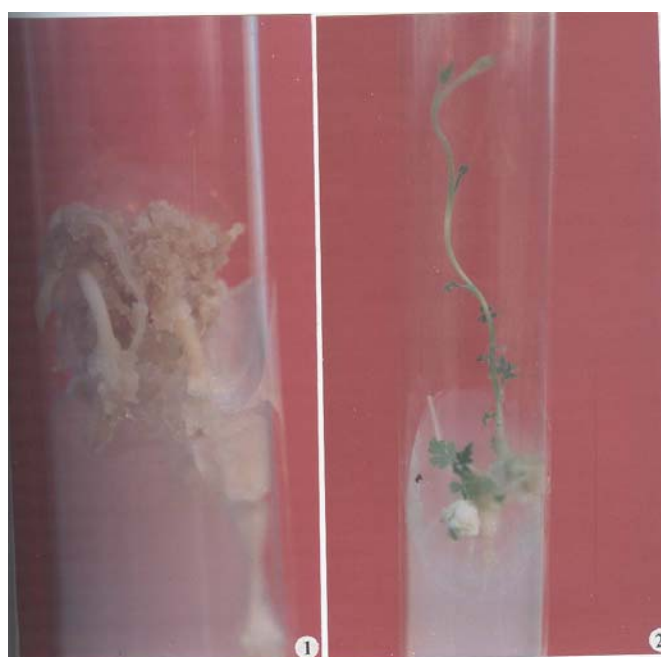


Figure 1: Stem explant treated with 0.25 EMS and cultured on MS + 1.0 mg/l 2,4 – D + 1.0 mg/l NAA producing dark brown callus. **Figure 2:** Callogenesis and direct shoot induction from explant cultures treated with 0.25 EMS cultured on MS + 1.0 mg/l Kn + 1.0 mg/l L – glutamic acid.

It was observed that the growth responses, color and friability of the callus were strongly influenced by the physical and chemical mutagen. (Table 1&2). The following variations were observed during *in vitro* mutagenesis. 1. Color of the callus varies from white to yellowish, white to dark brown and greenish to dark green. 2.

Callus may be friable, compact and hard compact. 3. rooting at higher doses like 5 and 10kR. 4. Variations in regenerated plants like shoot buds.

In – vitro techniques and radiation induced mutagenesis have been broadly recommended to improve plants (Anonymous, 1986, 1987; Maluszynski *et al.*, 1995). The effect of gamma rays in tissue culture has been reported in different plant materials (Degani and Pickholz, 1973; Rao and Narayana Swamy, 1975; Werry and Stoffelson, 1981).

In the present investigation *in – vitro* mutagenesis was used to study the effect of gamma irradiation on callus induction and morphogenesis. The lower doses of irradiation promoted the callus growth and higher doses decreased it. Such findings were reported by Venkateshwaran and Partanen (1966) in tobacco. George and Rao (1980) reported the doses like 2, 3 and 4 kRs favoured callus and multiple shoot induction. Proliferation of the tissue was more marked in the segments that had received 5 kR (Sham Rao and Narayana Swamy, 1975). Mustafa *et al.*, (1993) reported the effect of gamma irradiation on morphogenesis from different explants of *Momordica charantia*. The effect of gamma irradiation on growth and cytology of carrot tissue culture was reported by Bassam, Safadi and Simon (1990).

Morphogenesis was observed after two sub – cultures in *Bryonopsis*. Effect of irradiated sucrose on morphogenesis was also studied (Amirato and Steward, 1969). It was suggested that radiation induced organogenesis result from inactivation of auxin by radiation removing the inhibition of bud formation. In the present study variations in leaf, floral characters, induction of callus and shoot formation were observed. This finding is supported by Mustafa *et al.*, (1991).

Table 1: Effect of Gamma Irradiation on Rhizogenesis from Nodal Explant on Ms Bap, 2,4 – D, Naa, Tdz, L – Glutamic Acid and Cm in *Bryonopsis Laciniosa* (L.) Naud.

Dose (kR)	% of cultures with growth response	Morphogenetic response
Control	65	Roots + Shoots
1	60	Initiation of callus
2	62	White hard callus
3	65	Friable callus
4	70	Initiation of roots
5	85	Callus with 1-2 roots
10	90	Plant regeneration
15	65	Compact callus
20	NR	NR
25	NR	NR
30	NR	NR

Data scored at the end of 4 weeks of culture; NR = No Response.

Table 2: Morphogenetic Response Of Stem Explant Derived From Ems (0.25%) Treated Seedling Of *Bryonopsis Laciniosa* (L.) Naud On Ms With 1.0 2,4 – D +1.0 Naa And 1.0 Kn + 1.0 L – Glutamic Acid

Stem Treatment (h)	% frequency of Growth response	Morphogenetic response
Control	70	Callus + roots +shoots
0.1% EMS		
6	55	Callus with small shoot buds
12	40	Dark brown callus
18	30	Brown callus
24	15	Death of callus
0.25% EMS		
6	40	Induction of shoots
12	25	Browning of callus
18	15	Hard compact callus
24	NR	NR

Data scored at the end of 6 weeks of culture; Data scored at the end of 6 weeks of culture; NR = No Response.

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