

Effect of gamma rays on different explants of callus treatment of multiple shoots in *Cucumis melo* cv. Bathasa

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Abstract: A mutagenesis programme was carried out using physical mutagens (gamma rays) on *Cucumis melo* cv. Bathasa. In irradiated seeds the number of shoots formed in the lower doses was significantly higher than the controls. Decrease in the number of shoots and shoot bud formation was observed with higher doses in all the explants studies. There was complete lethality in the 10, 15 and 20 kR doses. In irradiated stem cultures the maximum number of shoots were observed in 2 kR. The number of shoots decreased with increasing doses of irradiation. At higher doses of 4 and 5 kR, light green compact callus was formed in almost all the explants. The effect of lower doses of gamma irradiation on shoot bud formation and rooting efficiency from nodal explants cultured on MS + 2.0 mg l⁻¹ L-glutamic acid + 0.5 mg l⁻¹ BAP, stimulation of shoot and root induction were studied. The number of shoots and root lengths decreased with the increasing dosage of irradiation. The irradiated callus was grown on solidified MS medium with containing 2.0 mg l⁻¹ BAP + 1.0 mg l⁻¹ IAA. There was a significant stimulation of growth in the callus at lower doses. At higher doses like 15 and 20 kRs growth was drastically reduced.

Key words: Gamma rays, Stem explant, Nodal explant

Introduction

In the recent years, there has been a major thrust in the application of physical and chemical mutagens in plant tissue culture. Application of physical and chemical mutagens in tissue culture has been reported (Bottino, 1975). The greatest advantage of *in vitro* systems is the ease of treating great number of cells with mutagenic agents, which has provided more effective and rapid results than conventional techniques King (1984) reported mutagenesis in *in vitro* as an important field for crop improvement. A combination of explant irradiation and *in vitro* regeneration is most effective for manifestation of variants (Novak and Micke, 1987).

Cucumis melo cv. Bathasa resembles cucumber and is used as a vegetable. The fruits are slender and elongated, the length varying from a few inches to about 3 ft. They are pale or dark green in colour, smooth or ridged, with soft downy hairs covering the skin when tender. Seeds are smaller than those of the musk melon. The seeds are cooling diuretic and are said to be beneficial in painful micturition and suppression of urine. This variety is cultivated both as a hot weather crop and as a rainy season crop. The primary aim of this study has been to gain some knowledge about the genotypic differences for callus initiation and high frequency plant regeneration from long term callus cultures of *C. melo* cv. Bathasa. It grows on any kind of soil, but thrives best on well-manured rich loamy soils with abundant water supply. Transmission of induced modification in flower colour and stipule character had also been reported earlier in other mutant lines derived through gamma rays irradiation in the same variety (Talukdar and Biswas, 2002).

Materials and Methods

The stem raised from seeds treated with 1, 2, 3, 4, 5, 10, 15 and 20 kR irradiation produced a number of multiple shoots. 4 kR or

5 kR is maximum potent in inducing most number of multiple shoots. In the present studies, the induction of multiple shoots were reported from stem and nodal explants after gamma rays treatment. The stem raised from control seeds could produce only callus on Murashige and Skoog's medium (MS) with different supplements which was regenerated into a single shoot. The isolated *in vitro* raised shoots of 1-2 cms long, rooted profusely on MS medium with Benzyl amino purine (BAP) (2.0 mg l⁻¹) + Naphthalene acetic acid (NAA) (1.0 mg l⁻¹) within 15 days resulting in the formation of complete plantlets. For gamma rays treatment, the seeds of *C. melo*, were presoaked for 6 hr in distilled water (Sample containing 200 seeds, pH 7.0) and freshly papered in 0.1 M phosphate buffer for 4 hr at room temperature and then washed thoroughly in running tap water for 6 hr to remove the traces of 10 kR, 15 kR and 20 kR doses. The parent seeds treated in similar way but without mutagen served as control. The observations were recorded on yield and various yield traits on 20 normal looking plants selected randomly in each progeny both in 15 kR and 20 kR doses. The genetic make up of the crosses was altogether different from the parental lines. The perusal of clustering pattern indicated that the grouping was not influenced by the place of origin, rather genetic background influenced their clustering behaviour (Yadav *et al.*, 2004).

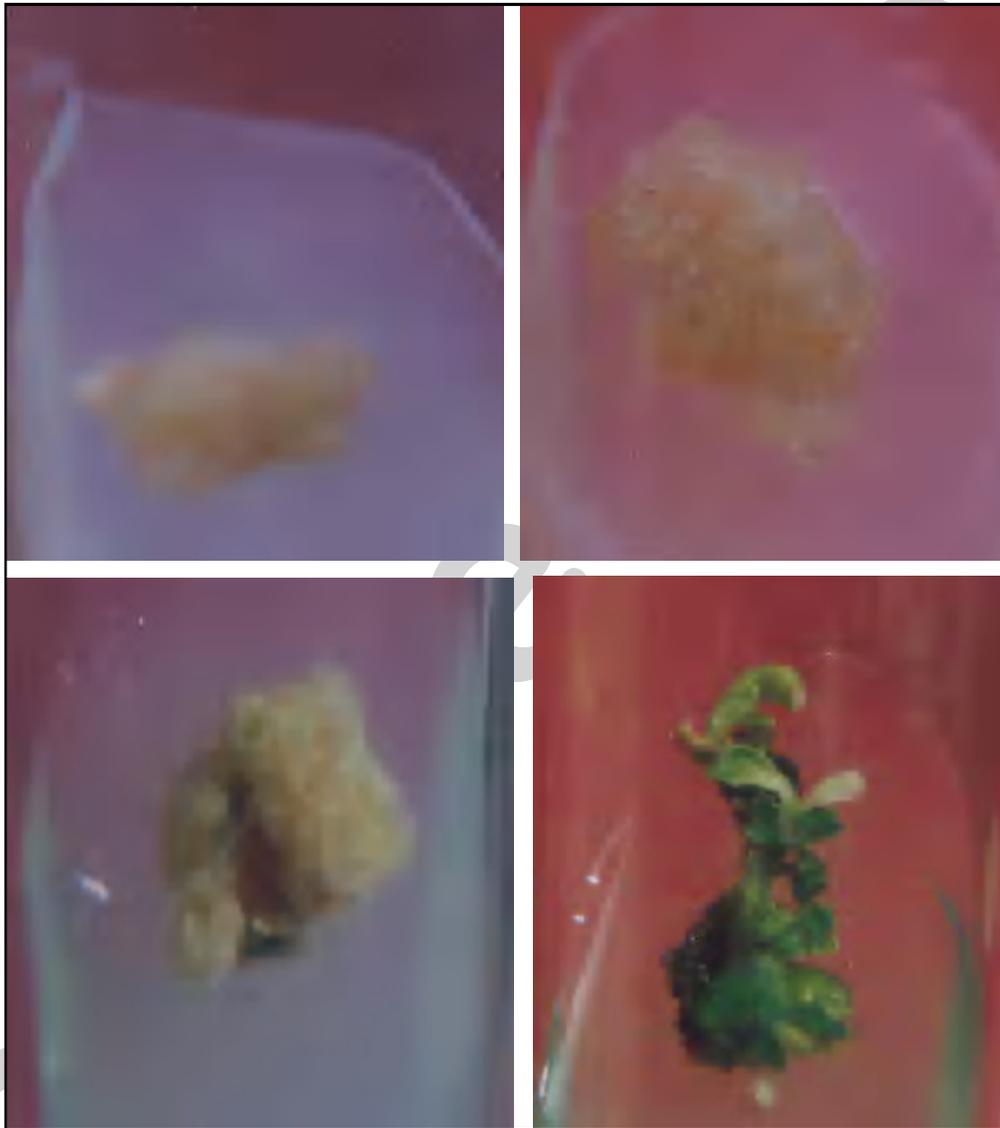
Results and Discussion

In the present study, relatively small being derived only from the higher doses of mutagens employed in the study (Mahana *et al.*, 1990) but several chlorophyll and morphological mutants in cowpea, upon treatment with sodium azide were also observed. Generally in any mutation breeding experiment the convention is to use both, higher and lower doses in order to have a reasonable chance of recovery of mutations. Murugan and Subramanian (1993), observed similar shifts in the mean values in cowpea varieties upon radiation



Table - 1: Effect of gamma rays in callus treatment of multiple shoots in *Cucumis melo* cv. Bathasa

Dose of irradiation (kR)	Stem (No. of shoots)	Nodal (No. of shoots)	Callus growth
Control	2 - 3	3 - 4	Normal callus growth
1	2 - 4	2 - 3	Better callus growth
2	3 - 4	2 - 4	Better growth
3	4 - 7	3 - 6	Better growth
4	5 - 7	4 - 6	Better greening of callus
5	3 - 4	2 - 3	Growth in abundance
10	5 - 6	4 - 5	Slow growth
15	3 - 5	3 - 4	Slow growth
20	2 - 4	3 - 5	Slow growth

**Plate - 1:** Effect of gamma rays on stem explants of *Cucumis melo* cv. Bathasa

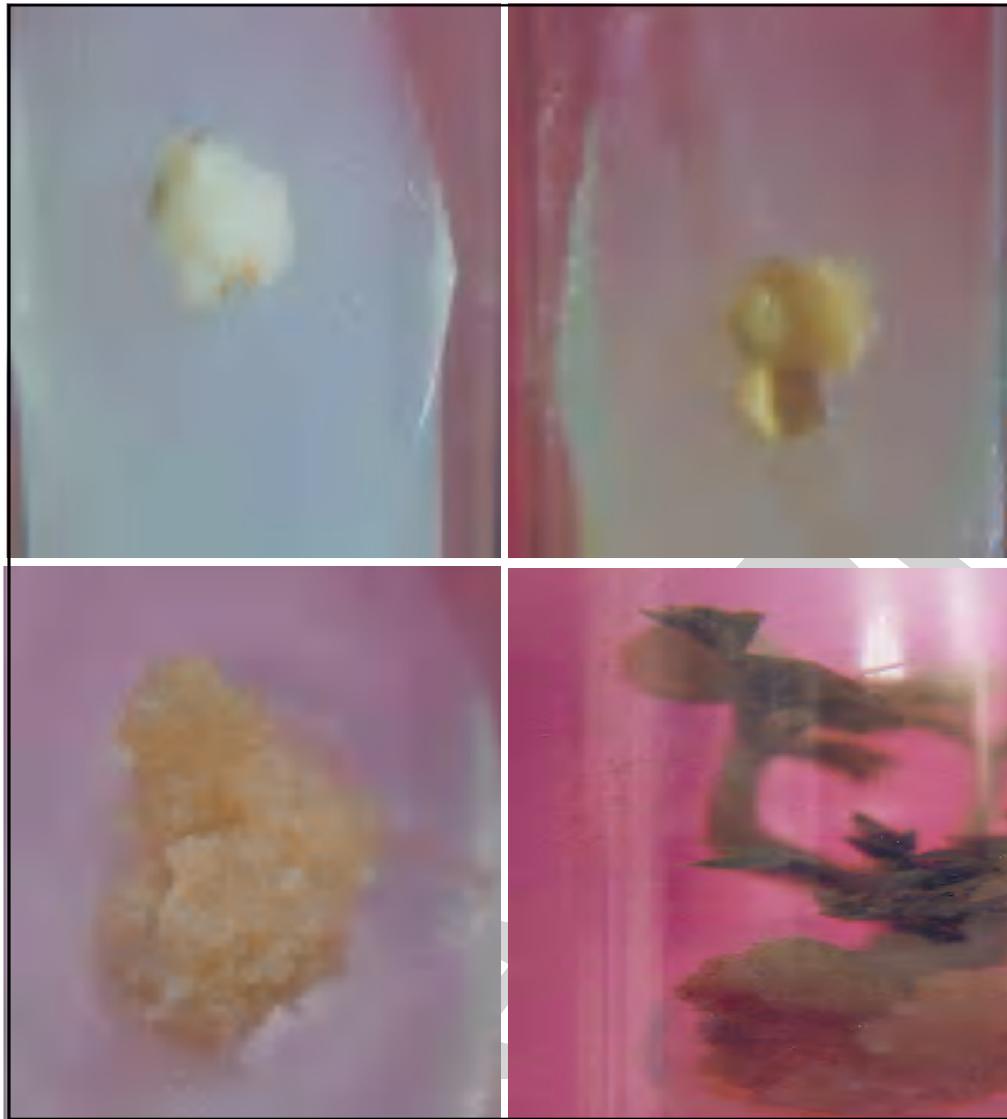


Plate - 2: Effect of gama rays on nodal explants of *Cucumis melo* cv. Bathasa

with gamarays. Genetic parameters of variation were estimated for all the characters studied in order to asses the possibility of improvement and also to determine selection criteria for improvement of seed yield per plant.

Mutation breeding is relatively a quicker method for improvement of crops (Kharkwal *et al.*, 2004). Many physical and chemical mutagens have been used for induction of useful mutants in rice (Singh *et al.*, 1998). The number of shoots and root lengths decreased with the increasing dosage of irradiation. The irradiated callus was grown on solidified MS medium containing 2.0 mg l⁻¹ BAP + 1.0 mg l⁻¹ IAA. There was significant stimulation of growth in the callus at lower doses like 15 kR and at 20 kRs growth was drastically reduced. The frequency and spectrum of different types of morphological mutants were recorded as per procedure at various developmental stages, particularly from flowering to maturity period

(Kharkwal, 2000). Mutagen (gamma rays) treated two different (Stem and nodal) explants were tested for their effectiveness. The lower doses induced callus and greening of callus in stem cultures (Plate-I, Fig. 1-3). The doses 4kR or 5kR induced high frequency of multiple shoots. The higher doses viz., 10, 15 and 20 kR induced excessive callus growth and browning of callus. The maximum number of shoots obtained at dose of 4 and 5 kR and they ranged from 12-16 from nodal cultures. The higher doses of 20 kR reduced the number of shoots and only greening of callus and doses beyond that totally suppressed the formation of shoots from stem and nodal explants (Table 1, Plate II, Fig. 1-3). Morphogenetic responses were more positive from the explants than from the callus. With the increase in the level of BAP (2.0 to 4.0 mg l⁻¹), the percentage of stem and nodal explants producing shoots was raised (Plate-I, Fig. 4, Plate-II, Fig.4). The maximum number of shoots on the stem and nodal explants were observed at 4.0 mg l⁻¹ BAP or 5.0 mg l⁻¹ kinetin,

but at higher level of BAP or kinetin, the formation of callus had taken place and the number of shoots per explants was reduced. From the result of present study it may be inferred that relatively higher doses of mutagens are effective in inducing not only macro mutations but also micromutations in desirable direction at least in *C. melo* cv. Bathasa.

The percentage response of stem and nodal explants decreased steadily with the increase of concentration / duration. The maximum number of callus and shoots were formed at 15kR and 20 kR doses. In irradiated stem and nodal explants the number of shoots formed due to lower doses was significantly higher than in the controls. Decrease in the number of shoots and shoot bud formation was observed with higher doses in all the explants studied.

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