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Biological Effect of gamma irradiations on in vitro culture of Stevia rebaudiana

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ABSTRACT

Current investigation was carried out to study effects of gamma irradiations on in vitro culture of S. rebaudiana. The dosages used were 5, 10, 15, 20, 25 and 30 kR (Kiloradians). Explants were selected from plants, derived from gamma irradiated seeds were subjected for callus induction, callus proliferation and somatic embryogenesis on the MS medium fortified with 2.5 mg/l 2,4-D. Differences in the morphology and growth rate of callus were recorded. High dose of Gamma irradiation showed necrosis of explants. The somatic embryogenesis percent decreased with increasing dosage of gamma radiations. Somatic embryo generation was significantly affected by higher doses of gamma rays. This study also indicates that plants produced from seeds irradiated with 5 - 10 kR gamma rays influences callus induction and growth. Explants obtained from plants, grown from gamma irradiated seeds with higher doses such as 25 kR and 30 kR delays callus induction. The data on Days to callus induction, growth rate and somatic embryogenesis, indicate that low dose of gamma radiation is effective for the production of variability in Stevia plants.

Key word : Stevia, gamma irradiation, in vitro culture, Callus induction

Introduction

The medicinal plant Stevia (Asteraceae family) produces sweet steviol glycosides in the leaves which can be used as a natural alternative to artificial sweetener (M. V. Chalapathi, S. Thimmegowda, 1997). The leaves of Stevia contains Stevioside as a main sweet component, which is a diterpenoid tetracyclic glycoside with a sweetening power ranging from100-400 times higher than sucrose. It has been used in a wide range of processed foods as a substitute for conventional sugars or artificial dietetics especially in Japan (C. M. Ferreira and W. Handro, 1988). Experimental mutagenesis has been applied in various plants. Mutations are the tools used to study the nature and function of genes which are basis of plant growth and development (A.K. Adamu and H. Aliyu, 2007). Poor seed germination is one of the factors limiting large-scale cultivation of Stevia (C. C. Shock 1982). Gamma irradiation was found to increase plant productivity. It was reported by Jaywardena and Peiris (1988), that gamma rays represent one of the important physical agents used to improve the characters and productivity of many plants such as rice, maize, bean, cowpea and potato. The productivity can be enhanced in castor cultivation through level of gamma radiation (Sharma and Rana, 2007). The effects of pre-sowing seed treatments on the germination, emergence and survival of wheat had positive results (Basra et al., 2005). In vitro technique can be used for both seed and vegetatively propagated species. The present study was undertaken to induce physical mutations to assess the effect of gamma radiations on the plant. It is expected that the physical mutagens would induce variability. This study will provide information on effect of gamma irradiation on the in vitro culture of Stevia rebaudiana. Rapid mass propagation of Stevia is possible through somatic embryogenesis and organogenesis; it will enable large number of plantlets to be produced within short span of time. (Meenakshi Banerjee and Priyanka Sarkar, 2009). The experiment was carried out to find a specific dose of gamma radiation which affects callus induction through leaf explants obtained from S. rebaudiana plant obtained from gamma irradiated seeds. Development of a new variety of S.rebaudiana with higher content of steviol glycosides can be possible by gamma irradiations. Explants of a secondary metabolite producing plant, cultured in vitro, may retain the capacity to synthesize the compound identical to that in the intact plant. Tissue grown as callus masses can sometimes yield high amount of secondary metabolites (L. Sivaram and U. Mukundan, 2003). It is expected that the gamma radiations would induce variability. This study will provide information on the variation in the pattern of callus induction and somatic embryogenesis in S. rebaudiana. The experiment was carried out to find effect of gamma rays on callus induction and somatic embryo regeneration studies. Parameters used to screen biological effect gamma irradiations were callus induction percentage, morphology of callus, somatic embryo generation percent from leaf explants collected from plants produced from gamma irradiated seeds.

Materials and methods

Seeds of S. rebaudiana were obtained from Jamna Biotech, Pune, India. Dry seeds of S.rebaudiana were eradicated with a 60Co sources emitting gamma rays at the Department of Physics, University of Pune, India. Seeds were divided into seven groups. First group did not have any treatment to serve as control, while the other six groups were irradiated with Gamma rays (5, 10, 15, 20, 25 & 30 kR).

Seeds were sown on the next day of irradiation. Leaf explants were selected from plants, derived from gamma irradiated seeds. The explants were prepared and sterilized with Bavistin (1% w/v) for 3 minutes. Explants were treated with 70% ethanol for 60 seconds and mercuric chloride (0.1% w/v) for 5 minutes followed by washings with sterile distilled water for 3 to 4 times to remove the traces of HgCl2. The explants were inoculated on MS medium (T. Murashige, F. Skoog, 1962) fortified with 3% sucrose and supplemented with 2.5 mg/lit of a 2, 4-D. The pH of the media was adjusted at 5.8 before solidifying the medium with 0.8% agar (Himedia). The cultures were incubated at 25 ± 1°C with a photoperiod of 16 h at 3000 lux light intensity of cool white fluorescent light. All the experiments were repeated twice with 10 cultures per treatment. Visual observation of culture was made every day and data were recorded after every week of inoculation for one month. Callus induction percent and growth rate of calli from each treatment was recorded considering number of days required for callus induction and proliferation.

Result and discussion

The mutagenic effect of different concentrations of gamma irradiations on callus induction and callus growth were examined. Differences in the morphology and growth rate of callus were recorded. High dose of Gamma irradiation showed necrosis of explants. The duration of callus induction significantly increased as compared to control. It was observed that at higher doses of gamma radiations mostly affects callus growth. In most of the treatments of gamma rays ranging from 5 kR to 15 kR, there was no significant callus growth.

Figure 1: Callus induction from Stevia leaf explants (5 KR)

Figure 2: Callus induction from Stevia leaf explants (15KR)

Figure 3: Callus induction from Stevia leaf explants (25KR)

5kR was found to be more effective and profuse callus was formed on MS medium supplied with 2, 4 D (2.5 mg/l). There was no significant callus growth of explants of 20 kR gamma irradiated S. rebaudiana plant. This study reveals that leaf explants delays callus induction of plants obtained from gamma irradiated seeds. Explants obtained from plants, grown from gamma irradiated seeds with higher doses such as 25 kR and 30 kR delays callus induction (Table 1).

Table 1: Effects of gamma irradiation on induction of Stevia callus from leaf explants

Gamma doses	No. of days	Callus Induction (%)	Growth Rate	Colour of callus	Nature of callus	Lethal Rate of explants (%)
0	7	64.0	+++	Greenish	Watery	0.0
5	8	76.6	+++	Greenish	Moist	0.0
10	7	80.0	+++	Greenish	Moist	0.0
15	10	65.3	++	Light green	Moist	10.0
20	15	53.6	+	Light yellow	Less moist	33.3
25	18	40.8	±	Yellowish	Less moist	33.3
30	23	16.0	±	Pale white	Less moist	70.0

This study also indicated that the lower doses of gamma irradiation influences callus induction and generation of somatic embryos. The somatic embryogenesis percent decreased with increasing dosage of gamma radiations. Somatic embryo generation was significantly affected by higher doses of gamma rays (Table 2).

Table 2: Effects of gamma irradiation on callus proliferation and somatic embryogenesis from leaf explants of Stevia

Gamma doses	Callus proliferation	Somatic embryos	Growth rate	Mature embryos(%)	Immature embryos(%)	Survival
0	+++	++	+++	26.5	73.5	96.0
5	+++	++	+++	23.3	76.5	88.4
10	++	+	+	17.0	83.0	65.0
15	+	+	+	15.6	84.4	61.3
20	±	-	-	0.0	0.0	0.0
25	-	-	-	0.0	0.0	0.0
30	-	-	-	0.0	0.0	0.0

Use of gamma irradiated seeds creates mutation in plants to improve their traits. These results indicate that plants obtained from gamma irradiated seed is more effective in the range of 5 to 15 kR for callus induction and proliferation as compared to explants taken from plants produced from seeds treated with 20-25 kR gamma rays. In ginger LD50 values reported by Giridharan and Balakrishnan (1992) were 1.5 to 2 kR and 1.0 to 1.25 kR as reported by Jayachandran and Mohankumar (1992). In contrast, Nwachukwu et al. (1994) reported a low dose of 0.87 kR as the LD50 for black ginger. The efficiency of mutant plant formation depends on various conditions such as pH, concentration of solution, presoaking in water, temperature and treatment duration. This type of study is also reported in turmeric micro-propagation. (Vidya M.M., 1989). As Seeds have high regenerative potential, it plays important role in mutagenesis. To increase production of the crop there is need to have a better understanding of its genetic background. Callus mostly introduces somaclonal variation induction. In vitro mutagenesis can be important technique for varietal improvements in plants. Chemical and physical mutagens have been utilized to widen the genetic base for breeding of many vegetatively propagated crops. S. K. Datta et.al. (2005) reported a quick method of In vitro mutagenesis for establishment of solid mutant in chrysanthemum. It was reported that physical mutagens like gamma rays and X- rays are extensively used instead of chemical mutagens (Nymbom, 1961). The useful mutant isolated through in vitro mutagenesis, in the present study, need to be tested further on a wider scale to establish any changes in stevioside content of treated plants and also to assess its performance in later generations.

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