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IN VITRO EFFECT OF BASE ANALOGUE AND 50 HZ RADIATIONS ON SECONDARY METABOLITES PRODUCTION IN *TRIGONELLA FOENUM - GRAECUM* L. AND *PROSOPIS CINERARIA* L. DRUCE.

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Hypocotyl derived calli of *Trigonella foenum - graecum* L. and *Prosopis cineraria* L. Druce were selected against the influence of 5 - azacytidine (5-azaC), a base analogue and 50 Hz radiations (a non-ionising extremely low frequency electromagnetic radiations) for their production of secondary products (trigonelline, diosgenin, β-sitosterol). Both agent caused diminutive effect on secondary metabolite production and some positive implications were also noticed during the investigation.

Keywords : Base analogue; *In vitro*; 50 Hz radiations ; Secondary metabolites.

Introduction

In recent years, considerable interest has been generated in nucleoside analogue of DNA, 5-azacytidine (5 - azaC) since they have a remarkable ability to alter the gene expression in the form of physiological process, altered growth patterns, differentiation and morphological variations in the form of new patterns[1]. Non-ionising extremely low frequency (ELF) electromagnetic (EM) radiations having a frequency of 10^{12} emitted by domestic powerlines, mobile phones, computer, televisions which are responsible for causing various diseases that are threatening to human population like cancer, skin diseases and deformities of brain[2].

Invariably, the influence of 5-azaC has been studied in higher plants[3] as well as in cell culture [4-6]. Few researchers have also studied their possible effects on different plant species *in vivo* [3,7] and *in vitro* [8,9].

Nevertheless, no work, has been done on the effect of 5 - azaC and 50 Hz radiations in production of plant secondary metabolites under tissue culture process. The purpose of study is to investigate wheather base analogue and 50 Hz radiations have any potentiality to alter the production of secondary metabolites under tissue culture process. *Trigonella foenum - graecum* L. and *Prosopis cineraria* L. Druce produce many secondary metabolites such as trigonelline, diosgenin (in *T. foenum - graecum*) and β-sitosterol (in *P. cineraria)* which are used in medicine and pharmaceutical preparations.

Material and Methods

The seed material of *T. foenum - graecum* and *P. cineraria* obtained from Tabiji Agricultural Farm, Ajmer, were surface sterilized with mercuric chloride (0.1%) and washed thrice with sterile distilled water. The seeds were germinated under aseptic conditions on MS[10] (Murashige and Skoog's) basal medium gelled with 0.8% agar-agar. The callus cultures were initiated from hypocotyl portion of explants excised from 10-15 days - old seedlings germinating on MS basal medium. Then the hypocotyl portion was kept on MS medium supplemented with IAA, 10 mg/l ; IBA, 3 mg/l ; BAP, 3mg/l ; kinetin, 0.04 mg/l used for *T. foenum - graecum* and 2 MS medium (double micronutrients); 2, 4 - D, 2.5 mg/l ; NAA, 0.5mg/l ; BAP, 1 mg/l used for *P. cineraria.* The pH of the medium was adjusted to 5.6 prior to adding of agar; the cultures were maintained at 26 ± 1^0C. After about 30 days and 45 days respectively as one passage of growth, the callus was subcultured on fresh MS medium for another subculturing growth.

Approximately, 100 mg (fresh weight) calli tissue clump of both plant species of each were transferred to MS medium for 5-azaC treatments and 50 Hz exposures. In one set, static callus cultures were administered with freshly prepared aqueous solution of 5-azaC (5, 10, 15 and 20 μm), after 3rd, 5th and 7th day for continuous supply of 5-azaC, in first week of callus growth. Similarly, for another set of experiment of ELFEMR radiations calli were exposed to 50 Hz radiations for various durations (5, 7, 9 ad 13 min) in a specially designed apparatus in first week of callus growth.

For both plant species, 5-azaC treated and 50 Hz exposed calli were maintained upto four passages (120 and 180 days). No further treatment and radiation exposure was provided.

Estimation of secondary metabolites trigonelline, diosgnenin and β-sitosterol in control as well as treated and exposed cultures was done with standard protocols [11-13].

Table 1. Effect of 5-azacytidine and 50 Hz radiations on secondary metabolites of *Trigonella foenum-graecum* L. and *Prosopis cineraria* L. Druce on *in vitro* cultures.

Concentrations/ Exposure duration	Secondary metabolic contents (mg/g/dw) Trigonelline		Diosgenin		Sitostoral	
	Culture Period (Days) 30 mean ± SE	120 mean ± SE	30 mean ± SE	120 mean ± SE	45 mean ± SE	180 mean ± SE
Control	1.78 ± 0.15	1.76 ± 0.14	2.63 ± 0.09	2.62 ± 0.02	1.38 ± 0.14	1.36 ± 0.20
5-azaC						
5 m	1.67 ± 0.15	1.67 ± 0.13	2.87 ± 0.13	2.85 ± 0.12	1.25 ± 0.21	1.30 ± 0.15
10 m	1.64 ± 0.20	1.68 ± 0.16	2.52 ± 0.14	2.60 ± 0.12	1.11 ± 0.11	1.25 ± 0.17
15 m	1.60 ± 0.14	1.65 ± 0.21	2.33 ± 0.19	2.55 ± 0.20	1.02 ± 0.14	1.20 ± 0.14
20 m	1.52 ± 0.11	1.62 ± 0.14	2.10 ± 0.10	2.53 ± 0.13	0.98 ± 0.14	1.19 ± 1.22
'r' =	-0.8401	- 0.4820	-0.8431	-0.8431	-0.6432	-0.5012
$\bar{X}x$ =	1.401	1.820	2.432	2.621	1.212	1.232
50 Hz exposures						
5 min	1.95 ± 0.04	1.90 ± 0.11	2.55 ± 0.21	2.60 ± 0.11	1.58 ± 0.17	1.56 ± 0.12
7 min	1.68 ± 0.12	1.72 ± 0.03	2.47 ± 0.10	2.61 ± 0.02	1.13 ± 0.04	1.29 0.16
9 min	1.60 ± 0.20	1.70 ± 0.14	2.30 ± 0.17	2.59 ± 0.07	1.20 ± 0.22	1.27 ± 0.07
13 min	1.51 ± 0.14	1.71 ± 0.13	2.11 ± 0.15	2.60 ± 0.15	1.20 ± 0.14	1.30 ± 0.19
'r' =	-0.8432	-0.8920	-0.9821	-0.5437	-0.7321	-0.6731
$\bar{X}x$ =	1.441	1.648	2.438	2.442	1.073	1.172

Reported values are mean ± SE of 3 replicates
Tabulated are for 4 d.f. at P = 0.05 is 0.811
'r' = Coefficient of correlation
$\bar{X}x$ = Percentage average of occurrence
SE = Standard Error

Results and Discussion

Secondary metabolic contents in callus cultures steadily decreased with an increase in the concentrations of 5-azaC and exposure of 50Hz radiations. Enhanced effect was observed in diosgenin production in 5-azaC treated series at low concentration and trigonelline, β-sitosterol contents in 50Hz exposed series at shorter duration of exposure which is also maintained upto last passages (Table 1). Detrimental effect was observed in all treated and exposed series till the last passage.

In the present experiments, after taking into account the combined mean values of percentage average of occurrence (Xx), maximum damaging effect was caused by 5-azaC followed by 50Hz exposures.

The statistically analyzed data (as per 'r' value at significant level P = 0.05) revealed that significantly negative correlation exists between concentrations of 5-azaC, exposure duration of 50 Hz and trigonelline / diosgenin / β-sitosterol contents.

In the present investigation both the treatments of short and long term effects on metabolites status of 5-azaC treated and 50 Hz exposed callus were observed. The observations support earlier researchers who have reported short term effects of 5-azaC on metabolite contents in callus culture. Vesely and Cihak[14] reported short term effect of 5-azaC treatment on inhibition of protein and pyrimidine synthesis. Similarly, Stafford *et al.*[15] observed 5-azaC treatments had short term effect on nicotine accumulation in *Nicotiana tabaccum* leaf explants culture. Arfmann *et al.*[16] not only recovered a new secondary metabolite from *Catheranthus roseus* cultures after 5-azaC treatment but also synthesis of new metabolite was maintained upto few passages. Chanabe *et al.*[17] have reported detrimental effect of weak electric pulses in cultured cells of sunflower. Rathore and Goldsworthy[8] have reported that application of weak electric pulse to tobacco callus stimulated the callus growth upto a certain duration of exposures. Present study shows that in both the plant species low concentration of 5-azaC and low duration of 50 Hz exposures increases the secondary metabolite production.

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