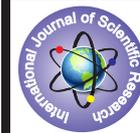


Evaluation of genotoxic potential of an agricultural soil sample of Nangli village of Amritsar (India) under rice cultivation



Biology

KEYWORDS : Soil contamination, Genotoxic potential, Allium cepa.

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ABSTRACT

Nowadays, the problem of soil pollution is increasing at alarming rates not only in developed countries but in developing countries as well. A number of genotoxic compounds have been discharged on to the lands which directly or indirectly find their way into various biological systems including the human beings. Upon reaching the human systems, these pollutants can not only cause direct toxicity but can also potentially damage the gene pool. Keeping this in mind, the present study was planned to evaluate the genotoxic potential of an agricultural soil sample collected from Nangli village of Amritsar under rice cultivation employing *Allium cepa* root chromosomal aberration assay (AIRCAA). Two types of treatments viz., in situ and root dip treatment were followed. Both the treatments resulted in different types of aberrations like laggards, vagrants, c-mitosis, delayed anaphase, stickiness (physiological aberrations) and chromosomal breaks, chromatin bridges (clastogenic aberrations). Root dip treatment induced 12.63 % of total aberrant cells at highest concentration (100%) whereas in situ treatment induced 12.03 % total aberrant cells.

Introduction

Soil is a complex physical, chemical and biological system providing support, water, nutrients and oxygen to the plants. 1-3 Agriculturally, it serves as a reservoir of nutrients and water for crops, provides mechanical anchorage and favorable tilth. Apart from these, it acts as a connecting link between inorganic, organic and living systems of the ecosystem. 4 But in recent years, the problem of agricultural soil pollution has increased to greater extent due to unremitting use of pesticides, inorganic fertilizers, heavy metals etc. 5-7 Therefore, the evaluation and remedial measures of these environmental toxicants is need of the hour. Various higher plant bioassays are being used to evaluate the genotoxicity of harmful chemicals in environmental complex mixtures. 8-10 Among various plant bioassays, *Allium cepa* root chromosomal aberration assay has been widely recommended for genotoxicity evaluation of soils by the scientists worldwide. 11-15 Considering the serious consequences of soil pollution on human health, the present study was aimed to evaluate genotoxic potential of an agricultural soil sample of Nangli village of Amritsar, Punjab (India) using *Allium cepa* root chromosomal aberration assay (AIRCAA).

Materials and methods

Collection of Soil Sample

Soil sample was collected from five different regions of an agricultural field of Nangli village, Amritsar, Punjab (India) under rice cultivation. Samples were pooled to denote the single sample of that area.

Estimation of genotoxic potential

Genotoxic potential of soil sample was estimated by using *Allium cepa* root chromosomal aberration assay.

Treatments

Uniform sized onion bulbs were purchased from local market and were peeled off. The primary roots were removed with the help of forceps without disturbing the root primordia. Onion bulbs were exposed to two modes of treatments viz., in situ and root dip treatments.

In situ treatment

Onion bulbs were exposed to soil samples contained in small earthen pots. After 24-36 h, when roots of 0.5-1 cm length emerged, root tips were washed thoroughly, cut and fixed in Farmer's fluid (3 : 1:: ethanol : acetic acid glacial). Root tips were squashed in aceto-orcein and slides were screened under microscope (Olympus CH20i) to study various types of chromosomal aberrations.

Root dip treatment

Preparation of soil extract

100 g soil was dissolved in 200 ml distilled water (1 : 2 :: w/v) and kept on mechanical shaker for 12 h. Solution was filtered through Whatman no. 1 filter paper and the filtrate was considered as soil extract. Different concentrations of soil extract viz., 20%, 40%, 60%, 80% and 100% were prepared by serial dilutions assuming original filtrate as 100%.

Treatment with soil extract

Onion bulbs were allowed to root in distilled water contained in Couplin jars. After 24-36 h, 0.5-1 cm roots were treated with different concentrations (20%, 40%, 60%, 80% and 100%) of soil extract (1 : 2, w/v ; soil : water) contained in Couplin jars. After 3 h treatment, root tips were washed thoroughly, cut and fixed in Farmer's fluid (3 : 1:: ethanol : acetic acid glacial). Root tips were squashed in aceto-orcein and slides were screened under microscope (Olympus CH20i). Cells were scored for different types of chromosomal aberrations.

Negative control

Garden loamy soil was used as the negative control in both treatments i.e. in situ and root dip treatment.

Calculations

Percent aberrant cells were calculated by using the formula:

$$\text{Percent aberrant cells} = \frac{\text{No. of aberrant cells}}{\text{No. of dividing cells}} \times 100$$

Level of significance was checked at $p \leq 0.05$ level of significance using Student's 't' test for in situ treatment and using one way ANOVA (analysis of variance) for root dip treatment.

Results

Both modes of treatments viz., in situ and root dip treatments showed significant genotoxic potential in *Allium cepa* roots by inducing various types of chromosomal aberrations (Fig. 1 and Table 1). The aberrations were classified into physiological and clastogenic ones. The spectrum of physiological aberrations included laggards, vagrants, stickiness, delayed anaphases, c-mitosis, abnormal metaphases and abnormal anaphases while clastogenic aberrations included chromatin bridges and chromosomal breaks. The root dip treatment of *Allium cepa* roots under different concentrations of soil extracts resulted in a dose dependent increase in chromosomal aberrations ranging from

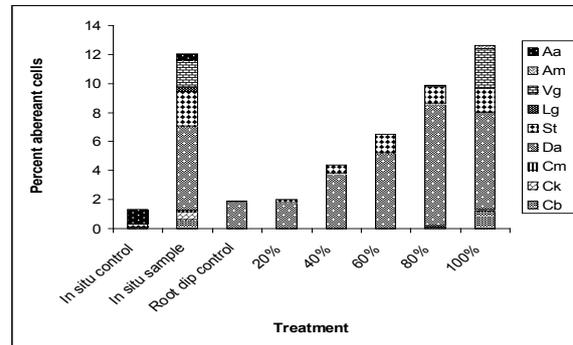
2.52% at 20% of soil extract to 12.63% at 100% of soil extract. The frequency of physiological and clastogenic aberrations ranged from 2.52% to 11.43% and 0.1% to 1.19%, respectively, for root dip treatment. For in situ treatment, frequency of total chromosomal aberrations was 12.03% consisting 10.84% physiological aberrations and 1.18% clastogenic aberrations.

Discussion

Assessment of the ecological and genetic impact of soil pollution is a matter of growing environmental concern since contaminants of soil can enter human populations through pathways such as inhalation of dust which contains these compounds, ingestion of plants that uptake the compounds from soil and leaching of the compounds from soil to ground water and surface water. Many earlier studies have reported the contamination of agricultural soils from various sources (application of pesticides, use of inorganic fertilizers, industrial effluents etc.). Monitoring of genotoxic effects of soil by a cytological assay and mutagenicity assay provides an alternative to chemical analysis because such assays give measure for relative toxicity i.e. the effects of bioavailable fractions of interacting pollutants present in soil (complex organic mixture). There are many reports on estimation of genotoxic potential of different kinds of soils using a number of plant bioassays. The results of this present investigation reveal genotoxic nature of the soil sample studied which is a clear indication of continuously growing pollution of soil ecosystem.

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Aa- Abnormal anaphase/s; Am- Abnormal metaphase/s; Vg-Vagrant chromosome/s; Lg Laggard/s; St-Stickiness; Da-Delayed anaphase/s; Cm-c-mitosis; Ck-Chromosomal break/s; Cb-Chromatin bridge/s.

Fig. 1: Spectrum of different types of chromosomal aberrations induced in root tip cells of Allium cepa following the treatment with soil sample from an agricultural field of Nangli village, Amritsar (India) under the cultivation of rice.

Table 1. Genotoxic potential of agricultural soil sample of Nangli village of Amritsar, Punjab (India) under rice cultivation in Allium cepa root chromosomal aberration assay.

Treatment	Conc.	TC	TDC	PA									CA			TAC		
				Cm	Da	St	Lg	Vg	Am	Aa	Total cells with PA	Cb	Ck	Total cells with CA	PA+CA			
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
In situ control	-	4265	900	0	2	3	0	0	0	0	14	1.56	0	0	0	0	14	1.56
In situ treatment	-	4775	913	1	54	22	3	17	0	4	101	10.84	6	5	11	1.18	112	12.03*
Root dip control	-	4384	944	17	2	0	0	0	0	0	19	2.013	0	0	0	0	19	2.010a
Root dip treatment	20%	5587	914	0	17	6	0	0	0	0	23	2.52	0	0	0	0	23	2.520a
	40%	5638	924	0	35	6	0	0	0	0	41	4.437	0	0	0	0	41	4.440b
	60%	5410	937	2	49	12	0	1	0	0	64	6.830	0	0	0	0	64	6.830c
	80%	5578	977	1	82	12	0	7	0	0	102	10.44	1	0	1	0.1	103	10.540d
	100%	5104	918	1	62	15	0	25	2	0	105	11.43	11	0	11	1.19	116	12.630e

F-ratio (5, 48) = 149.788, HSD = 1.493

TC: Total number of cells; TDC: Total number of dividing cells; Cm: c-mitosis; Da: Delayed anaphase; St: Stickiness; Lg: Laggard/s; Vg: Vagrant/s; Am: Abnormal metaphase; Aa: Abnormal anaphase; Cb: Chromatin bridge/s; Ck: Chromosomal break/s; PA: Physiological aberrations; CA: Clastogenic aberrations; TAC: Total aberrant cells

*statistically significant at p≤0.05

Values followed by the same letter do not differ significantly (p≤0.05)

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