Genotoxicity Evaluation of Soil Sample from Agricultural Field under Wheat Cultivation

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**Abstract:** The agricultural soil has been polluted through various sources like application of pesticides, inorganic fertilizers, industrial effluents and wastewater discharges. Furthermore, different heavy metals like arsenic, cadmium, copper, cobalt, lead, manganese, mercury, nickel and zinc are also reported to add on the agricultural soil pollution. All these contaminants have threatened the life of various organisms including human beings. Considering this, the present study was planned to evaluate the genotoxic potential of an agricultural soil sample collected from Nagli village of Amritsar, Punjab, India under wheat cultivation employing *Allium cepa* root chromosomal aberration assay (AIRCAA). Two types of treatments viz., *in situ* and root dip treatment were followed. The root tips were squashed in aceto-orcein to prepare slides and slides were observed for various types of chromosomal aberrations. Both the treatments resulted in different types of aberrations like laggard/s, vagrant/s, c-mitosis, delayed anaphase/s and stickiness (physiological aberrations) and chromosomal break/s and chromatin bridge/s (clastogenic aberrations). It was found that the sample was significantly genotoxic. Among two types of treatments used, root dip treatment was found to be more effective resulting 12.81 % of total aberrant cells at highest concentration (100%) as compared to *in situ* treatment which resulted in 9.2 % cells with chromosomal aberrations.

**Key words:** Soil pollution · Pesticides · Chromosomal aberrations · *Allium cepa*

**INTRODUCTION**

Population explosion, urbanization and industrialization have not only depleted various natural resources but also have caused the pollution of every component of environment be it air, water or soil [1-3]. Among various types of pollutants, soil pollution is of serious concern because soil is the most important factor to sustain life on earth. Soil is healthy if it is consists of roughly 40% mineral, 23% air, 6% organic and 8% living organisms. However, in recent years, pollution of agricultural soils has increased to greater extent due to continuous use of pesticides and inorganic fertilizers which ultimately pose ill health effects to living beings [4-6]. Some of the pollutants like heavy metals are documented to cause severe damage to the gene pool [2, 3, 7, 8]. Hence it is obligatory not only to analyze the soil characteristics but also to explore their potential hazards employing various bioassays.

Various higher plant bioassays are being used to evaluate the genotoxicity of harmful chemicals in environmental complex mixtures like soil matrices [3, 9-13]. Among various plant bioassays, *Allium cepa* root chromosomal aberration assay has been recommended for genotoxic evaluation of soils [1, 3, 10]. Considering the serious consequences of soil pollution on human health, the present study was planned to evaluate the genotoxic potential and physicochemical characteristics of an agricultural soil sample of Nagli village of Amritsar, Punjab, India using *Allium cepa* root chromosomal aberration assay (AIRCAA).

**MATERIALS AND METHODS**

**Estimation of Genotoxic Potential**

**Chemicals and Make:** Various chemicals used to study the genotoxicity of an agricultural soil sample in *Allium cepa* assay were glacial acetic acid
(Thomas and baker); ethanol (Changshu Yangyuen Chemical, China); Orcein stain (Spectrochem) and hydrochloric Acid (Qualigen).

**Collection of Soil Sample:** Soil sample was collected from five different regions of an agricultural field of Nangli village, Amritsar, Punjab, under wheat cultivation. Samples were pooled to denote the single sample of that area.

**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 prepared. The 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.

**Allium cepa Root Chromosomal Aberration Assay:**

Onion bulbs were allowed to root in distilled water contained in coplin jars. After 24-36 h, 0.5-1 cm roots treated with different concentrations (20%, 40%, 60%, 80% and 100%) of soil extract (1: 2, w/v; soil: water) contained in coplin 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 to prepare slides and slides were screened under microscope (Olympus CH20i). Cells were scored for different types of chromosomal aberrations.

**Calculations:** Percent aberrant cells were calculated by using the formula:

\[
\text{Percent aberrant cells} = \left( \frac{\text{No. of aberrant cells}}{\text{No. of dividing cells}} \right) \times 100
\]

**RESULTS AND DISCUSSION**

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). The spectrum of various included laggards, vagrants, stickiness, delayed anaphases and c-mitosis (physiological aberrations) and chromatin bridge/s and chromosomal break/s (clastogenic). Some of the aberrant anaphases which could not be included in any one of the categories mentioned above were termed as abnormal metaphases/anaphases. 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 2.59% at 20% of soil extract to 12.81% at 100% of soil extract. The frequency of physiological and clastogenic aberrations ranged from 2.59% to 11.3% and 0.11% to 1.51%, respectively, for root dip treatment. For *in situ* treatment, the total chromosomal aberrations frequency observed was 9.2% with 8.31% physiological aberrations and 0.98% clastogenic aberrations. Among the two types of treatments studied, root dip treatment was found to be more effective producing 12.81 % of total aberrant cells at highest concentration (100%) as compared to *in situ* treatment where maximum of 9.2 % cells showed chromosomal aberrations.

![Fig. 1: Spectrum of different types of chromosomal aberrations induced in root tip cells of Allium cepa under the treatment of soil sample from an agricultural field of Nangli village (Amritsar).](image-url)
Many earlier studies have reported the contamination of agricultural soils due to various sources (application of pesticides, use of inorganic fertilizers, industrial effluents etc.). 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 [14]. 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 [1, 3, 7, 8, 12, 13,15].

REFERENCES