International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Allium cepa Root Chromosomal Aberration Assay: An Efficient Test System for Evaluating Genotoxicity of Agricultural Soil

Rajneet Kour Soodan[1], Jatinder Kaur Katnoria[2], Avinash Nagpal[3]*

[1, 2, 3]Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, 143005, Punjab, India
*Professor, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, 143005, Punjab, India

Abstract: ***Agricultural soils are increasingly becoming sinks for wide number of hazardous contaminants which ultimately result in pollution. Plant bioassays, because of their sensitivity and affordability, have been recommended as first-tier assays which enable the detection of contaminants. The present study involves genotoxicity of four soil samples collected from different regions of Amritsar employing chromosomal aberration assay in root tip cells of Allium cepa using in situ and root dip modes of treatments. The squash preparations from root tip cells of treated A.cepa bulbs revealed different types of chromosomal aberrations which were apportioned into physiological aberrations (c-mitosis, delayed anaphases, stickiness, laggards, vagrants) and clastogenic aberrations (chromosomal breaks, chromatin bridge and ring chromosomes). Frequencies of chromosomal aberrations induced by soil samples were higher than negative control. A few cells with c-mitosis, delayed anaphase, stickiness, bridges were observed whereas no instance of laggards, vagrants, abnormal anaphase, abnormal metaphase, breaks and ring chromosomes were found in negative control. Among physiological aberrations, percentage of delayed anaphases was maximum where as chromatin bridges dominated clastogenic aberrations.***

Keywords: Agricultural soil, *Allium cepa*, genotoxicity, chromosomal aberrations

1. Introduction

Soil, an important environmental medium, sustains life on earth and serves as a reservoir of nutrients, provides mechanical anchorage and favorable tilth. Apart from these, it acts as a connecting link between inorganic, organic and living systems of the world [1]. In recent decades, soil has been exposed to a number of pollutants by various natural and anthropogenic activities. These contaminants in soil has potential to pose severe health risk to humans through various routes of exposure such as direct ingestion, contaminated drinking ground water and food crops; dermal contact and through food chain. Soil ecosystems are so complex that the impact of these pollutants not only affect humans but also causes acute toxicity to various soil organisms, flora and fauna [2], [3]. Therefore, it is mandatory to evaluate agricultural soils for their potential risks in biological systems. The analytical approach does not consider mixture toxicity, nor does it take into account the bioavailability of other pollutants present in the soil. In this respect, bioassays provide an alternative because they constitute a measure for environmentally relevant toxicity i.e. the effects of bioavailable fractions of interacting pollutants present in a complex environmental matrix. Historically, plants have been a choice of research and constitute an important material for genetic tests to monitor environmental pollutants. Cytogenetic tests in plants are well established systems for screening and monitoring of genotoxicity, validated in international collaborative studies and demonstrated to be efficient test for monitoring the genotoxicity of environmental pollutants [4], [5]. Plant bioassays are relatively inexpensive; can be easily handled; more sensitive and simpler than other methods used for detection of genotoxicity of environmental pollutants. Plant roots are extremely useful in biological testing because root tips are the first to be exposed to toxicants dispersed in soil or in water [6]. Therefore, the root tip chromosomal aberration assays constitute rapid and sensitive methods for biomonitoring the extent of pollution and to evaluate the effects of toxic and mutagenic substances in the natural environment [7], [8]. Presently in India, the ongoing rigorous agricultural practices are pulling out the essential nutrients out of soil. The district Amritsar of Punjab (India), an agricultural land, is under intensive cultivation of wheat, rice and some vegetable crops. In order to have high yield, vast varieties of pesticides and fertilizers, both organic and inorganic are being used by the farmers, which ultimately result in soil and water pollution. Keeping in view the alarming consequences of contamination of agricultural soils of Amritsar, Punjab (India), the present study was planned to evaluate the genotoxic potential of different soil samples from agricultural fields of Amritsar

2. Materials and Methods

2.1 Study area and collection of samples

The study area in present investigation is district Amritsar, located in northwestern part of the Punjab (India). It lies between $31^0$28'30" to $32^0$03'15" north latitude and $74^0$29'30" to $75^0$24'15" east longitude with population of 2,490,656 and has 778 villages [9]. An extensive survey of Amritsar region was carried out during the initial phase of the study. The soil samples were collected from 4 agricultural fields of four different zones north east (NERF), south east (SERF), north west (NWRF) and south west (SWRF) under cultivation of rice crop during September, 2010.The soil samples were collected by digging soil to depth of 15-20 cm following random sampling method from 4-5 sites of each agricultural field and the samples were pooled to make single sample of that site. The samples were coded, brought to laboratory, dried

at room temperature for 72 h and finally ground to fine powder [10]. The soil samples were packed in poly bags until further investigation.

2.2 Estimation of genotoxic potential

2.2.1 *A.cepa* root chromosomal aberration assay

The common onion (*Allium cepa*) bulbs used for the present study were procured from the local market. Young onion bulbs of appropriate size were denuded by removing outer loose scales and scrapped at the bottom to expose root primordial. The genotoxic potential of rice cultivated agricultural soil samples was estimated using *Allium cepa* root chromosomal aberration assay by applying two modes of treatment, *viz., in situ* and root dip [11].

2.2.1.1 *In situ* treatment

In situ conditions were simulated by allowing the denuded onion bulbs to root directly in soil samples contained in pots for 24-48 h. Sand was used as negative control.

2.2.1.2 Root dip treatment

The soil extracts were prepared by suspending soil in distilled water in ratio of 1:2 (w/v) on shaker for 12 h and then filtered [10].The filtered extract was considered as 100 % and different concentrations (20, 40, 60, 80 and 100%) of soil extract were made. The denuded onion bulbs were placed on Couplin jars containing distilled water for rooting. After 24-48 h, the emerged roots of about 0.5 - 1.0 cm length were treated for 3 h by placing them on treatment jars containing different concentrations of each soil extract. Distilled water was used as negative control.

2.2.2 Cytological investigations

After treatment, the bulbs were thoroughly washed, root tips were plucked and fixed in Farmer's fluid (glacial acetic acid: ethanol:: 1:3) for 24 h and preserved in 70 % ethanol and stored at 4ºC till further use. For chromosomal analysis the fixed *Allium* root tips were hydrolyzed in 1N HCl with intermittent heating for 1 min. and then transferred to a watch glass containing mixture of 1N HCl and aceto-orecin (1:9). The root tips in watch glass were heated intermittently for 3 - 5 min, covered and kept aside undisturbed for 15-20 min [12]. Then the root tips were squashed in a drop of 45% glacial acetic acid. The slides were observed for different types of chromosomal aberrations. About 900-950 dividing cells from 9 root tips (~ 100 cells/ root tip) were scored. Photomicrographs were taken with the help of a digital camera fixed on microscope (Olympus) that was connected to a computer in order to transport images.

2.3 Statistical analysis

Experiment was conducted in triplicate, and data were analyzed by one-way analysis of variance (ANOVA). Significance was considered at $p \leq 0.05$.

3. Results

3.1. Genotoxicity by using *A.cepa* test

To estimate genotoxicity of soil samples *Allium cepa* root chromosomal aberration assay (*Al*RCAA) was used employing two modes of treatment *viz*., *in situ* and root dip. The frequencies of different types of chromosomal aberrations induced by all the soil samples during both the modes of treatments are given in Table 1 and Table 2. Different kinds of induced aberrations were apportioned into physiological aberrations attributable to spindle abnormalties and clastogenic aberrations attributable to direct action on chromosomes and physiological aberrations attributable to spindle abnormalties. The spectrum of physiological aberration included c-mitosis, delayed anaphase, laggard and vagrant chromosomes while that of clastogenic aberrations included chromosomal breaks, chromatin bridges and ring chromosomes (Fig.1). The squash preparations from root tip cells of control *A. cepa* bulbs revealed a large number of dividing cells at different stages of mitosis. During *in situ* mode of treatment chromosomal aberrations in root tip cells treated with different soil samples were found to be higher as compared to control (sand) which showed 4.14%. Among the soil samples studied, sample SWRF showed maximum (14.84 %) percentage of total chromosomal aberrations followed by SERF with 12.75%, NWRF with 12.20% and NERF with 11.24%. Delayed anaphases and chromatin bridges were the most common effects which dominated physiological and clastogenic aberrations, respectively. During root dip mode of treatment, the squash preparations from root tip cells of control (distilled water treated) *A. cepa* bulbs have shown total chromosomal aberrations of 3.46%. All the soil samples collected showed dose dependent increase in chromosomal aberrations with increase in concentration of soil extract. The total aberration frequency including both physiological as well as clastogenic aberrations for different soil extract concentrations (ranging from 20% to 100%) of soil samples NERF, SERF, NWRF and SWRF ranged from 10.13% - 18.56%, 8.03 % - 18.32%, 6.29% - 13.49% and 12.26% - 18.96%, respectively. The frequency of cells with delayed anaphase was found to be maximum followed by c-mitosis. Cells with stickiness, laggards and vagrants were also seen. Chromosomal breaks, bridges and ring chromosomes constituted the spectrum of clastogenic aberrations (Table 2). The statistical analysis revealed that the frequencies of chromosomal aberrations at all concentrations tested differed significantly from the control.

Table 1: Genotoxicity of agricultural soil samples (*In situ* studies)

					Chromosomal aberrations in *A.cepa* root tip cells												
					Physiological aberrations (PA)							Clastogenic aberrations (CA)			TAC		
Sample code	TDC	Cm	Da	Lg	St	Vg	Aa	Am	Total PA		Bg	Bk	Rc	Total CA		PA+CA	
									No.	%				No.	%	No.	%
Control	916	5	27	-	1	-	-	-	33	3.60	5	-	-	5	0.54	38	4.14
NERF	916	25	62	1	6	3	-	-	97	10.58	6	-	-	6	0.65	103	11.24*
SERF	925	20	71	1	4	-	-	-	96	10.37	22	-	-	22	2.37	118	12.75*
NWRF	918	23	63	-	4	3	1	-	94	10.23	18	-	-	18	1.96	112	12.20*
SWRF	923	13	88	1	13	1	-	-	116	12.56	19	-	2	21	2.27	137	14.84*

Control (Sand); TDC-Total no. of dividing cells; **Cm**-C-mitosis; **Da**-Delayed anaphase; **Lg**-Laggards; **St**-Stickiness**; Vg** - Vagrants; **Aa** –abnormal anaphase; **Am**- abnormal metaphase; **Bg**- Chromatin bridges; **Bk**-Chromosomal breaks; **Rc**-Ring chromosomes; **TAC -** Total aberrant cells (PA+CA).

Significantly different at $p \leq 0.05$

Table 2: Genotoxicity of agricultural soil samples (Root dip treatment)

			Chromosomal aberrations in *A.cepa* root tip cells														
			Physiological aberrations (PA)									Clastogenic aberrations (CA)			TAC		
Sample code@	Conc (%)	TDC	Cm	Da	Lg	St	Vg	Aa	Am	Total PA		Bg	Bk	Rc	Total CA		PA+CA	
										No.	%				No.	%	No.	%
Control		924	4	25	-	1	-			30	3.24	2	-	-	**2**	**0.21**	32	3.46
NERF	20	918	31	46	3	5	3	-	-	88	9.58	5	-	-	5	0.54	93	10.13
	40	911	10	73	2	14	3	-	-	102	11.19	22	-	-	22	2.41	124	13.61
	60	919	14	88	1	16	2	-	-	121	13.16	19	-	-	19	2.06	140	15.23
	80	921	20	94	1	22	5	-	-	142	15.41	15	-	-	15	1.62	157	17.04
	100	925	45	75	-	27	11	-	-	158	17.08	13	-	-	13	1.40	171	18.56
SERF	20	921	20	40	-	9	-	-	1	70	7.60	-	4	-	4	0.43	74	8.03
	40	918	24	48	1	12	-	-	-	85	9.25	-	7	-	7	0.76	92	10.02
	60	914	32	49	-	27	-	-	-	108	17.81	-	10	-	10	1.09	118	12.91
	80	919	34	56	1	36	2	-	-	129	14.63	-	12	-	12	1.30	141	15.34
	100	917	38	61	-	51	1	-	-	151	16.46	-	17	-	17	1.85	168	18.32
NWRF	20	921	10	40	-	8	-	-	-	58	6.29	-	-	-	-	-	58	6.29
	40	918	7	51	2	3	1	-	-	64	6.97	4	-	1	5	0.54	69	7.51
	60	917	13	64	-	9	-	-	-	86	9.37	-	-	-	-	-	86	9.37
	80	924	23	69	1	5	-	-	-	98	10.60	3	-	-	3	0.32	101	10.93
	100	919	31	74	2	12	1	-	-	120	13.05	4	-	-	4	0.43	124	13.49
SWRF	20	913	57	47	1	3	1	-	-	109	11.06	-	3	-	3	0.32	112	12.26
	40	914	59	60	-	3	-	-	-	122	13.34	-	5	-	5	0.54	127	13.89
	60	919	58	72	-	2	-	-	-	132	14.36	2	7	-	9	0.97	141	15.34
	80	929	61	76	1	4	-	-	-	143	15.39	2	12	1	15	1.61	158	17.00
	100	928	64	89	-	7	1	-	-	160	17.24	1	15	-	16	1.72	176	18.96

Control (Distilled water); TDC-Total no. of dividing cells; **Cm**-C-mitosis; **Da**-Delayed anaphase; **Lg**-Laggards; **St**-Stickiness**; Vg** –Vagrants **Aa** –abnormal anaphase; **Am**- abnormal metaphase; **Bg**- Chromatin bridges; **Bk**-Chromosomal breaks; **Rc**-Ring chromosomes; **TAC**- Total Aberrant Cells (PA+CA).

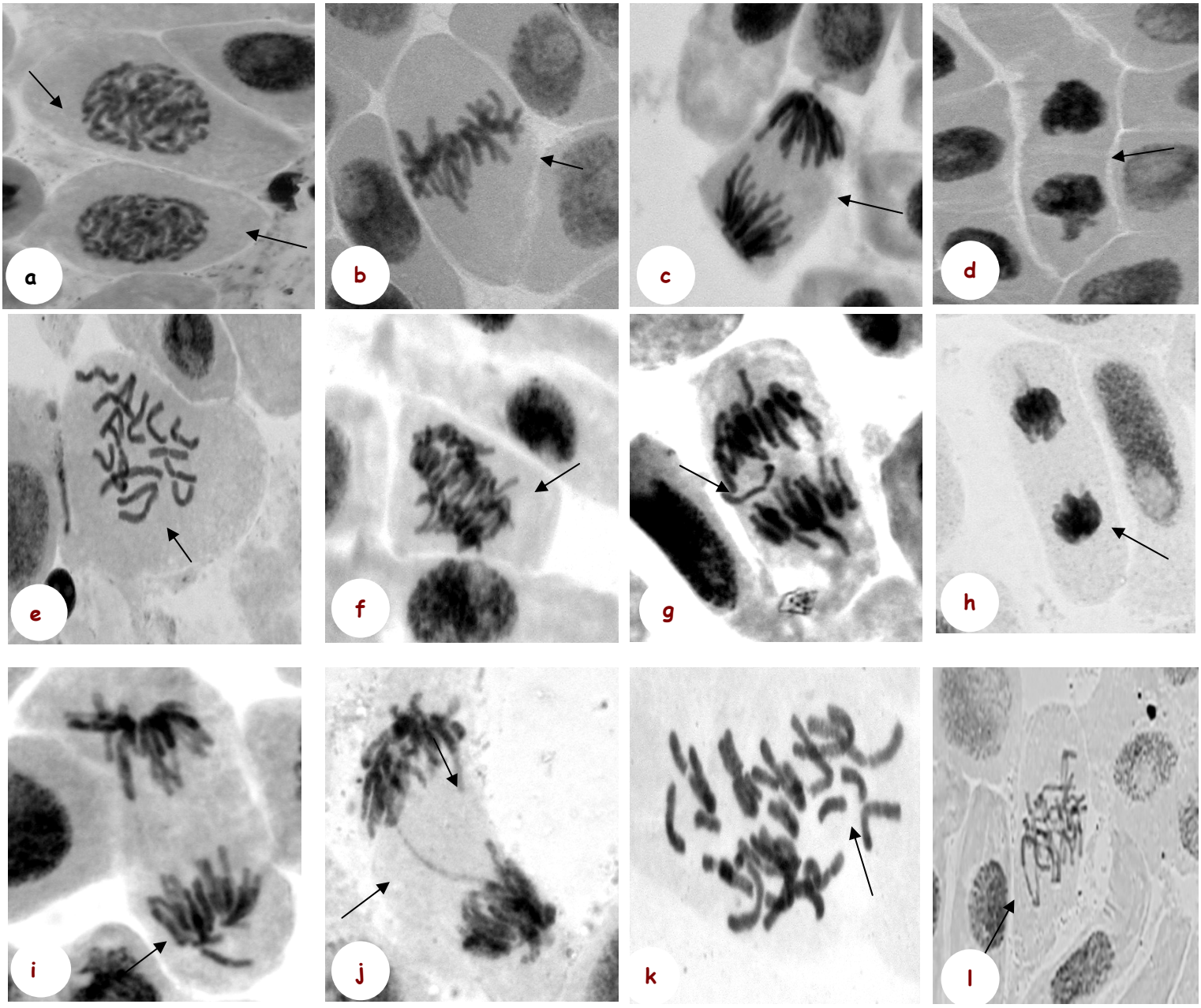

Figure 1: Representative photomicrographs of root tip cells of *Allium cepa* showing normal cells (a-d) : prophase (a), metaphase (b), anaphase (c), telophase (d),; physiological chromosomal aberrations (e-i): c-mitosis (e), delayed anaphase (f), laggard (g), stickiness (h) , Vagrant chromosome (i) and clastogenic aberrations(j-l): chromatin bridge (j), chromosomal breaks (k), ring chromosome(l).

4. Discussion

Chromosomal aberrations are considered as end result of genotoxic effects of various physical and chemical agents and are also estimates of exposure of various organisms to different physical and chemical agents [13]. The present study revealed genotoxicity of soil samples in terms of occurrence of frequency of chromosomal aberrations in both the modes of treatments. Delayed anaphase and c-mitosis were the most common effects followed by chromosomal breaks and bridges. The term c- mitosis was coined by Levan [14] to describe the effect of some chemicals which act in a fashion similar to that of colchicine which prevents the assembly of spindle microtubules by dissociating disulphide bonds. Delayed anaphase configuration has two anaphasic groups of chromosomes lie close to each other near equatorial plate. Chromosomal breaks are considered to involve the DNA molecule responsible for linear continuity of the chromosome and may be due to unfinished or misrepair of DNA [15]. The formation of anaphasic chromatin bridges are due to may be attributed to unequal exchanges resulting in formation of dicentric chromosomes which are pulled equally to both poles at anaphase [16]. According to Al- Najjar and Soliman [17], in addition to unequal translocation or inversion of chromosome segments, the formation of chromatin bridges are attributable to chromosome stickiness and subsequent failure of free anaphasic separation. Genotoxicity of the soil samples in the present study could be attributed to use of vast varieties of both organic and inorganic pesticides and

fertilizers by the farmers, which ultimately result in soil pollution. Apart from this, the direct use of sewage sludge, industrial wastes and waste water to agricultural land as source of plant nutrients aroused serious concern as they are known to contain many toxic metals along with useful nutrient elements. Some studies have reported different types of mitotic and chromosomal abnormalities showing genotoxic potential of contaminated soil from other regions of the world [18], [19]. Our results are in consistence with some earlier study of Dragoeva et al. [20] who evaluated genotoxic potential of agricultural soil and reported various chromosomal abnormalities like vagrant chromosomes, chromosomal fragments at anaphase and telophase and multipolar anaphases. Leme et al. [21] also assessed the genotoxicity of contaminated soil matrix by using *A.cepa* root chromosomal aberration assay and reported various chromosomal abnormalties in merismatic cells of *A.cepa.* In another report by Souza et al. [19] the clastogenic potential of polluted cultivated soils was assesed using the *A. cepa* root chromosomal aberration assay. Different types of chromosomal abnormalities observed in *A. cepa* root cells included anaphases with chromosome loss and chromosomal adherence, multipolar anaphases, chromosomal breaks and bridges.

5. Conclusions

The present study clearly indicates significant genotoxic effects of agricultural soil. It also helps us to acquire better understanding of soil contamination in a comprehensive manner in order to evade potential risks linked with contaminated agricultural soils and associated food chains. The study also suggests that response of *Alium cepa* genetic material can be used to evaluate the effects of potential genotoxic and cytotoxic substances in the environment.

References

[1] M. Fried, The soil plant system: In relation to inorganic nutrition, Elsevier Science, London, 2012.
[2] C.A. Edwards, "Assessing the effects of environmental pollutants on soil organisms, communities, processes and ecosystems," European Journal of Soil Biology, pp. 225-23, 2002.
[3] S.Feng, Z. Cao, Y. Yang, G. Wei, and X.M.Wang, "Polycyclic aromatic hydrocarbons, heavy metals, and genotoxicity of the suburban soils from Guangzhou, China," Polycyclic Aromatic Compounds, 33, pp. 501-518, 2013.
[4] S. Turkoglu, S, "Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L." Mutation Research, 626, pp. 4-14, 2007.
[5] Z.A. Alam, S. Ahmad, and A. Malik, Genotoxic amd mutagenic potential of agricultural soil irrigated with tannery effluents at Jajmau (Kanpur), India. Archives of Environmental Contamination and Toxicology, 57, pp. 463-476, 2009.
[6] G. Fiskesjo, "The Allium test- an alternative in environmental studies: The relative toxicity of metal ions," Mutation Research, 197, pp. 243-260, 1988.
[7] S.T. Matsumoto, M.S. Mantovani, M.I.A. Malaguttii, A.L. Dias, I.C. Fonseca, and M.A. Marin-Morales, "Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root-tips," Genetic and Molecular Biology, 29, pp. 148-158, 2006.
[8] A. Rodriguez-Ruiz, V. Asensio, B. Zaldibar, M. Soto, and I. Marigómez, "Toxicity assessment through multiple multiple endpoint bioassays in soils posing environmental risk according to regulatory screening values," Environment and Soil Pollution, 2014, doi:10.1007/s11356-014-2915-7.
[9] G. Kaur, B.P. Singh, and A.K. Nagpal, "Phenology of Some Phanerogams (Trees and Shrubs) of Northwestern Punjab, India," Journal of Botany, 2013. doi:10.1155/2013/712405.
[10] G.L.Cabrera, and D.M.G. Rodriquez, Genotoxicity of soil from farmland irrigated with wastewater using three plant bioassays, Mutation Research, 426, pp. 211-214, 1999.
[11] A. K. Sharma, and A. Sharma, Chromosome techniques: theory and practice, Butterworths, London, 1980.
[12] G. Fiskesjo, "The *Allium* test as a standard in environmental monitoring," Hereditas, 102, pp. 99-112, 1985.
[13] R. Pohren, C. Thatiana, and V.M.F. Vargas, "Investigation of sensitivity of the Allium cepa test as and alert system to evaluate the genotoxic potential of soil contaminated by heavy metals," Water Air Soil Pollution, 224, pp. 1460-1470, 2013.
[14] A. Levan, "The effect of colchicne on root mitosis in Allium," Hereditas, 24, pp. 471-486, 1938.
[15] H.J.S. Evans, Molecular mechanism in the induction of chromosome aberrations, In: D. Scott, B.A. Bridges, and F.H. Sobels, (eds.), Progress in Genetic Toxicology, p.57, Elsevier/North- Holland and Biomedical Press, Amsterdam, 1977.
[16] K. Sax, and H.J. Sax, "Possible hazards of some food additives, beverages and insecticides," Idengaku zasshi, 43, pp. 89-94, 1968.
[17] N.R. Al-Najjar, and A.S. Soliman, Cytological effects of herbicies I. Effect of 2, 4-D and 2,4,5-T on meiotic cells of wheat and two related species," Cytologia, 47, pp. 53-61, 1980.
[18] F. Masood, and A. Malik, "Cytotoxic and genotoxic potential of tannery waste contaminated soils," Science of the Total Environment, 444, pp. 153-160, 2013.
[19] T.S. Souza, F.A. Hencklein, D.F. Angelis, and C.S. Fontanetti, "Clastogenicity of landfarming soil treated with sugar cane vinasse,"Environmental Monitoring and Assessment, 185, pp. 1627–1636, 2013.
[20] A. Dragoeva, V. Kalchevai, and S. Slanev, "Genotoxicity of agricultural Soils after one year of conversion period and under conventional agriculture," Journal of Applied Science and Environment Management, 13, pp. 81-83, 2009.
[21] D.M. Lemea, T. Grummt, R. Heinze, A. Sehr, S. Renz , S. Reinel, D. Palma de Oliveirac, E.R.A. Ferrazc, M.R. Rodrigues de Marchi, M.C. Machado, G.J. Zocolo, M.A. Marin-Morales, "An overview of biodiesel soil pollution: Data based on cytotoxicity

and genotoxicity assessments," Journal of Hazardous Materials, 199, pp. 343-349, 2012.

Authors Profile

Rajneet Kour Soodan is Ph.D. Scholar at Department of Botanical and Environmental Sciences at Guru Nanak Dev University, Amritsar.

Dr. Jatinder Kaur Katnoria is Assistant Professor at Department of Botanical and Environmental Sciences at Guru Nanak Dev University, Amritsar

Dr. Avinash Nagpal is Professor Department of Botanical and Environmental Sciences at Guru Nanak Dev University, Amritsar.