MINI REVIEW

Assessment of Cytotoxic and Genotoxic Potential of Heavy Metals in Plants: A Review

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Abstract

Heavy metal stress is one of the major problems affecting the agricultural productivity of plants. Soil is contaminated by heavy metals, such as, Cd, Cr, Pb, Hg, and As. Plants suffer from oxidative stress upon exposure to heavy metals and lead to cellular damage. Heavy metals induce clastogenecity, aneugenicity, recombinogenicity, gene mutation, and DNA damage. There are several genetic endpoints that can be used as biomarkers of cytotoxicity and genotoxicity, e.g., comet assays, micronuclei frequency, meiotic analysis, mitotic analysis, and chromosomal aberrations. Plants are very useful in environmental monitoring and assessment as they provide a very wide range of genetic endpoints. They may be used as biosensors of the genetic toxicity of environmental pollutants.

Keywords: Cytotoxic, Genotoxic, Heavy metals, Xenobiotics.

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Introduction

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these heavy metals (Tchounwou *et al*., 2012 Kumar *et al*., 2020). The present study is of great concern because plants provide agricultural and medicinal plants for human and livestock. Plants are very important from a commercial product point of view. They are used for biomonitoring and phytoremediation of environmental pollutants. Plants are good bioindicators of heavy metals because they play a significant role in food chain transfer and in defining habit (Knasmuller *et al*., 1998; Grant, 1999).

There are various sources to which plants are exposed include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources. The present review provides an analysis of heavy metal occurrence, their exposure to plants, and consequently cytotoxicity and genotoxicity.

Contamination of agricultural soil by heavy metals has become a critical environmental concern due to their potential adverse ecological effects. Plants exposed to high levels of heavy metals cause a reduction in photosynthesis, water uptake, and nutrient uptake. As per the United States Environmental Protection Agency (USEPA) regulation, eight heavy metals, *viz*., Pb, Cr, As, Zn, Cd, Cu, Hg, and Ni are enlisted as the most pervasive heavy metals in the environment (Selvi *et al*., 2019). These heavy metals at elevated concentrations exert deleterious effects on organisms (Rahman *et al*., 2019; Gupta *et al*., 2020a,b). The uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to plants and human health. The contamination of heavy metals stimulates the production of reactive oxygen species (ROS) that leads to cellular damage. Heavy metals are converted to derivatives by the metabolic pathways of the biological system and these derivatives are reactive with DNA (Bhat *et al*., 2011). DNA is nature's most widely used long term information storage system. There are many types of DNA damage that impact replication by DNA polymerase (Lindahl, 1993). Generation of hydroxyl and superoxide radicals indicate the outburst of ROS, which could be synergist for increased membrane lipid peroxidation promulgating cytogenotoxicity (Adhikari *et al*., 2017) and damage of proteins and nucleic acids. Inhibition of DNA replication, gene expression, disorganization of the microtubular cytoskeleton in interphase and mitotic cells, and finally cell division arrest (Liue *et al*., 2009a,b; Roy, 2019).

Heavy metals cause damage to the structural, enzymatic, and non-enzymatic components of the plant cell, resulting in loss of cell viability and thereby, negatively impacting plant growth and development (Dutta *et al*., 2018). Such conditions, in turn, lead to genome instability and thus, eventually severely affecting plant health and crop yield.

Genetic Endpoints

Genetic endpoints in plants exposed to xenobiotics could be considered as prominent applicability of plants as bimonitoring tool for environmental contamination. These genetic endpoints provide accurate, rapid, and low-cost bioassay tool for ecotoxicological study. There are several genetic endpoints that can be used as biomarkers of genetic toxicity, e.g., comet assay, mitotic, and meiotic analysis frequency of chromosomal aberrations, and micronuclei test (Table 1).

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Table 1: Genotoxicity of metals in plants with specific genetic toxicological endpoints

Assay systems	Metals	Chromosome aberrations		Micro-nuclei	Sister chromatic exchange	Comet assays	References
		Aneugenic	Clastogenic				
Allium cepa	As	+	+				Gupta *et al*. (2018)
	Cd	+					Mishra (1997)
	Cr	+		+			Gupta *et al*. (2012, 2018)
	Hg	+	+				Mishra (1995)
Cicer arietinum	Cd		+			+	Roy (2019)
			+				Siddiqui (2015)
Eichhornia crassipes	Cd			+			Mishra *et al*. (2007)
	Cr			+			Mishra *et al*. (2009)
	Pb			+			Pratap *et al*. (2006)
Hordeum vulgare	Ni	+	+				Mishra and Singh (1999)
Lathyrus sativus	Cd	+	+				Adhikari *et al*. (2017)
Vigna radiata	Cr	+	+				Srivastava *et al*. (2014)
Vicia faba	Cd		+			+	Roy (2019)
	Cd				+		Panda and Panda (1996)
Tradescontia clones	Cr			+			Knasmuller and Fenech (2019)
	Ni			+			Knasmuller and Fenech (2019)

Comet Assay

Comet assay is a simple, most common sensitive, versatile, and reliable method for measuring DNA strand breaks. The assay has an application in testing genotoxicity and monitoring the environment with genotoxins. This assay has been used by several workers for DNA damage assessment (Bhat *et al*., 2011). Plant comet assay has been used to study the genotoxic impact of heavy metals. It provides information about cellular responses including oxidative stress, cell division, or cell death. The plant comet assay is a very important ecotoxicological approach. The prominent meristematic zone and direct contact of roots with their contaminated surrounding established the application of plant comet assay in the environment monitoring aspect (Santos *et al*., 2015).

Meiotic Analysis

Heavy metals affect the pairing of homologous chromosomes. Various types of meiotic lesions, such as, univalents, multivalent, laggard, bridges, stickiness, stray bivalent, disturbed polarity, and precocious separations have been recorded. Such anomalies are induced due to breakage or defective spindle which leads to imbalance daughter cells. Somatic, as well as, reproductive cells are affected by heavy metals. The most conspicuous effect of heavy metals on the plant is growth inhibition which is correlated with cell division.

Heavy metals induce a mutagenic effect. Heavy metals may be used as a mutagen to create genetic and phenotypic variation in plants which could be selected as useful and viable mutants and can be recommended to mutation breeding programs (Shahwar *et al*., 2018).

Heavy metals have deleterious meiotic effects. Meiotic recombination generates much of the genetic variability in sexually reproducing species and is known to be a highly conserved pathway. Environmental stress, such as, heavy metals changes the pattern of recombination in both models and crop plants. Heavy metals affect cellular processes meiosis being particularly vulnerable (Sidiqui, 2015; Fuchs *et al*., 2018).

Mitotic Analysis

The toxic effect of different heavy metals on the root tip cells of plants has been investigated by several workers. The degree of cell damage in the root tips is associated with the amount of heavy metals absorption and accumulation of heavy metals. Heavy metals disturb the mechanisms controlling the organization of microtubule (MT) cytoskeleton and tubulin assembly processes. These toxic effects result in a decrease in the mitotic index and stimulation of abnormal mitotic division. Heavy metals induce several chromosomal aberrations, such as, C-mitosis, anaphase bridge chromosome stickiness. Heavy metals disrupt metaphase and anaphase spindles, impairing chromosome segregation (Shi *et al*., 2014).

Heavy metals inhibit cell division and expansion, resulting in inhibition of root growth and the appearance of stunted roots (Zhang *et al*., 2009). Some damage induced by heavy metals can inhibit DNA repair mechanisms by competition with certain ions which are essential for DNA polymerase (Cao *et al*., 2014; Shi *et al*., 2014).

It shows a strong mitodepresive effect of heavy metals in the meristematic cells. Different types of chromosomal anomalies are also induced by heavy metals. The induction of chromosomal aberrations indicates aneugeneicity, clastogenicity, and mutagenicity. These types of chromosomal damage may induce cancer in somatic cells. Clastogenicity cause chromosomal breakage, aneugenicity cause a daughter cell to have an abnormal number of chromosomes. DNA strand breaks mutation, structural and numerical aberrations of chromosomes are known as genotoxicity, i.e., relevant to carcinogenesis. In this

way, the genotoxic mode of action has a very harmful impact on the organisms.

Micronuclei Frequencies

Micronucleus the most eminence is the name given to the small nucleus that forms whenever a chromosome or an arrangement of a chromosome is not incorporated into one of the daughter nuclei during cell division. It is a sign of genotoxic events and chromosomal instability. It indicates genomic damage. Many micronucleus assays have been developed to test the genotoxic events. The Tradescantia-Micronuclei (MN) [Tradescantia micronuclei (Trad-MN)] assay is suitable for the detection of the genotoxic effect of Heavy metals (HMs) and DNA damaging potential. A pronounced induction of the frequency of micronuclei in Trad-MN assay by HMs exposure has been observed (Knasmuller & Fenech, 2019).

Heavy metals may enter into the cell nucleus and may bind to purine and pyrimidine bases or proteins, denature spindle and cause MN formation as a result of a decrease in the chromosome number in the main nucleus (Jiang *et al*., 2001).

Higher plants are recognized as excellent indicators of cytogenetic and mutagenic effects of HMTs (Grant, 1999). Several HMTs, i.e., Ag, Cd, Cr, Cu, Hg, Mo, and Pb have mutagenic potential. It has been reported by Reutova (2017) using the *Crepis capillaries* L. plant test system.

Genetic toxicity of metals in plants, includes clastogenecity, aneugenicity, recombinogenicity, gene mutation, DNA damage, and repair and effect on cell cycle very well agree with results available from the mammalian or human system. Plants have their application as sentinel bioassay (Lower and Kendeall, 1990) in studying genecotoxicology of environmental pollution. Transgenic plants are also employed to biomonitor environmental genotoxicity (Kovalchuk *et al*., 2001).

Plant Bioassays

Plant genotoxicity assays are very useful in environmental monitoring and assessment (Houk, 1992). Many plants have a long and low number of chromosomes that are excellent test systems with a wide range of genetic endpoints from gene mutation to mitotic and meiotic chromosomal aberrations and DNA damage (Grant, 1999). Genotoxicity test results based on plant assays have shown a positive correlation with that of mammalian and non-mammalian assays (Chauhan *et al*., 1999). Plant assays are relatively easy and inexpensive to work with. Plant assays are unique in the sense that they can be employed to evaluate genotoxicity under a wide range of environmental conditions that include *in situ* monitoring (Sandhu & Lower, 1989). Some plants, *viz*., *Allium cepa* (2n = 16), *Glycine max* (2n = 40), *Hordium vulgare* (2n = 14), *Tradescantia clones* (2n = 12), *Vicia faba* (2n = 12), and *Zea mays* (2n = 20) are well worked out assays system that provides well-defined genotoxicity endpoints (Panda & Panda, 2002).

Conclusion

Plant assays can be used for the study of mutation, mitotic, and meiotic chromosomes, as well as, cell division, aberrations, micronucleus, sister chromatic exchange (SCE), and comet assays that evaluate DNA damage. As plants rapidly respond to alteration in osmotic pressure, temperature, mechanical stimulation or injury, water availability, and various xenobiotics, hence, these plants may be used as biosensors of the genetic toxicity of environmental pollution and for phytoremediation purpose.

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