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Review Article

Triticum aestivum Assay - A Useful Tool for Environmental Monitoring and Toxicity Assessment

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Abstract

The present review summarizes the literature data regarding the application of *Triticum aestivum* assay as an alternative method for toxicity assessment of environmental pollutants or potential therapeutic agents. Plant bioassays present several advantages among other biological assays (simplicity, low cost, rapid test activation, a wide array of assessment endpoints). They present a good correlation with animal and human cells models, and are a reliable tool for genotoxicity assessment. Furthermore, in the context of toxicology guidelines that promote the substitution of assays using animal models with other bioassays, genotoxicity assays using higher plants models have gained in popularity. The present review focuses on three major aspects regarding *Triticum aestivum* assay - its utility in environmental pollution monitoring, its application in genotoxicity assessment studies, and its application in phytotoxicity evaluation of nanomaterials.

Keywords: environmental pollutants; genotoxicity; nanoparticles phytotoxicity; plant assay; wheat

Introduction

The issue of exposure of living organisms to toxic compounds has been a matter of concern for a long time. From environmental pollution to foods and therapeutic agents (drugs, medicinal plants) with toxic potential, they all represent threats for the health and well-being of all living organisms. The observed impact depends on many factors, both species, and individual differences, and they can be short term effects, like immediate acute toxicity, or long term effects, such as genotoxicity, teratogenicity, mutagenicity, oncogenesis or phenotype changes (Wieczerzak *et al*., 2016).

In this context, a constant preoccupation for developing and improving existent toxicological studies is observed. Toxicological analyses can be divided into two groups. The first group aims towards the identification and quantification of environmental pollutants, using physical or chemical analyses, while the latter includes bioassays that measure the toxic impact on target organisms, without clearly identifying the compounds (Hassan *et al*., 2016). Currently, for toxicity assessment can be used cellular, bacterial, animal and plant bioassays, depending on the species of test organisms involved. Some bioassays are even available as toxkits, the test organisms being supplied in their cryptobiotic forms (cysts, seeds, microorganisms in a lyophilized form) and ready to be used after incubation under appropriate environmental conditions (Wieczerzak *et al*., 2016).

Genotoxicity assays represent an important component of toxicity assessment; they aim to identify compounds (drugs, food additives, pesticides, industrial chemicals of natural products) that have the ability to interact with nucleic acids and lead to chromosomal aberrations or changes in DNA structure (Sponchiado *et al*., 2016).

The applications of plant bioassays are not limited just to environmental monitoring. They can extend to medicine, the pharmaceutical or food industry, or other fields of activity, the main desiderata being to reduce the toxic risks associated with everyday products on living beings (Jitareanu *et al*., 2011; Wieczerzak *et al*., 2016).

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Plant toxicity assays are divided into two groups: macroscopic assays that assess germination and early seedling development, and microscopic assay, that analyze chromosome and nuclear alterations (Silveira *et al*., 2017). Quantitative determinations of various physiobiological parameters are also usually applied.

Methodology

A literature search was conducted using Scopus, Web of Science and Google Scholar. This review aims to give a comprehensive literature overview on the application of *Triticum aestivum* assay as a toxicity evaluation tool, considering especially the studies published in the last years.

Toxicity / Genotoxicity assessment using plant assays

Plant bioassays present several advantages among other biological assays: simplicity, low cost, rapid test activation, a wide array of assessment endpoints (germination rate, biomass weight, enzyme activity), they present a good correlation with other test models -animal models and human cells (Hassan *et al*., 2016; Silveira *et al*., 2017).

Plant assays are also a reliable tool for genotoxicity assessment. Several international organizations have recommended the use of higher plants for the detection of mutagens (the Royal Swedish Academy of Sciences, the Council of the Environmental Mutagen Society, the World Health Organization, the Swedish Board of the Protection of the Environment). Furthermore, the Gene-Tox program (launched by the US Office of Toxic Substances and the US Environmental Protection Agency) concluded that higher plants are a suitable tool for the detection of genotoxic contaminants (Lanier *et al*., 2015). To support this statement, several studies have proven the reliability of plant assay (Juchimiuk *et al*., 2006; Lanier *et al*., 2015; Palmieri *et al*., 2016; Reis *et al*., 2017).

Higher plants are used especially in biomonitoring of environmental contaminants and are considered excellent indicators of cytogenetic and mutagenic effects caused by different pollutants. The predominant species in plant toxicity assays is *Allium cepa*, but other species are also frequently used - *Nicotiana tabacum*, *Vicia faba*, *Hordeum vulgare*, *Triticum aestivum*, *Lactuca sativa* (Silveira *et al*., 2017).

These bioassays also have limitations. They are highly sensitive to heavy metals, but there are certain groups of genotoxic compounds (polycyclic aromatic hydrocarbons, nitrosamines, heterocyclic aromatic amines) that are difficult to detect in plant assay, plants lacking the enzymes necessary to activate these pro-mutagens (Mišík *et al*., 2019).

Besides ecotoxicology studies, plant assays can have applicability in other research areas as well, for example, genotoxicity assessment during the drug validation procedures. Genotoxicity of new drug candidates must be evaluated using *in vitro* and *in vivo* techniques. The comet assay and the micronucleus assay are the most commonly used, due to their robustness, sensitivity and statistical power to evaluate DNA breaks (Fig. 1). These tests can be performed on any eukaryotic cell population, including vegetal tissue (*Araldi et al*., 2015).

International regulations and guidelines for toxicity/genotoxicity evaluation using plants

Plant bioassays can represent a good alternative for toxicity and genotoxicity assessment. However, there are also several problems related to these assays. The most important is that, in some studies, discrepancies, and even contradictory results were obtained. Wang *et al*. (2016) identified one possible explanation for this situation - the different toxicity endpoints that were used in different studies - seed germination and seedling growth, enzyme activities, photosynthetic system, reactive oxygen species (signs of cytotoxicity) and genotoxicity evaluation (e.g., nucleic acid integrity and expression). Furthermore, different plant species can react differently to chemical

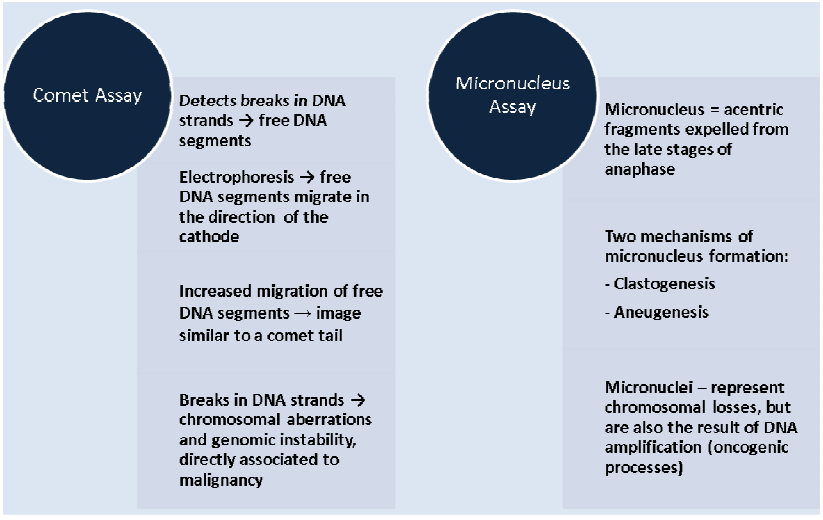

Fig. 1. The comet and the micronucleus assays

treatments. This is why the use of the adequate methodology in plant toxicology studies is essential. In this context, several International regulations and guidelines were elaborated (EPA, 2012a; EPA, 2012b; ISO, 2013; OECD, 2006) (Table 1).

Triticum aestivum is a species with great significance, one of the most important economic crop plants worldwide. In consequence, pollution with different xenobiotic agents can have severe repercussions on crop production and quality, but also human health. Therefore, wheat was used as an eco-toxicological indicator in many studies (Zhang Q *et al*., 2016; Qu *et al*., 2019b). *Triticum aestivum* can also be used as a genetic model to detect mutagens, by assessing DNA damage, materialized in chromosomal aberrations and abnormal mitotic cycles.

Table 1. International regulations and guidelines for toxicity/genotoxicity evaluation using plants

OECD Guidelines for the Testing of Chemicals. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test - assessment of the effects on seedling emergence and early growth of higher plants following exposure to the test substance in the soil	
Time	14 to 21 days after 50% emergence of the seedlings in the control group
Endpoints	- visual assessment of seedling emergence - biomass (fresh or dry shoot weight) - visual detrimental effects (chlorosis, mortality, development abnormalities, etc.).
Validity of the test	- respecting performance criteria for the control (at least 70% seedling emergence and 90% plant survival) - using a reference substance to verify the performance of the test - respecting the procedure: test design, test conditions (temperature, humidity, photoperiod), testing concentrations
Results	NOEC (no observed effect concentration) LOEC (lowest observed effect concentration) ECx/ERx (e.g. EC50/ER50 or EC25/ER25) values and related confidence limits for the endpoints
EPA Ecological Effects Test Guidelines OCSPP 850.4100: Seedling Emergence and Seedling Growth	
Time	14 days post-emergence of 50% of control plants, with the possibility of extension to 21 days post-emergence
Endpoints	- number (percent) of emerged plants - seedling survival - seedling length, and seedling biomass - qualitative phytotoxic effects
Validity of the test	- minimum seed germination standards for the control (at least 70%) and control survival rate (at least 90%) - at least five dosages should be tested - respecting test design and parameters (carbon dioxide level, humidity, photoperiods, temperature)
Results	NOEC (no observed effect concentration) LOEC (lowest observed effect concentration); EC25 / EC50 value for seedling emergence, and for survival; IC25 / IC50 value (with 95% confidence interval and standard error) for shoot length, and for shoot biomass.
EPA Ecological Effects Test Guidelines OCSPP 850.4230: Early Seedling Growth Toxicity Test (root and foliar exposure scenarios)	
Time	at least 14 days (after the germination of 50% of the control seeds)
Endpoints	- survival rate and morphology - length of roots, shoots and entire plant - weight of roots, shoots and entire plant
Validity of the test	- choosing the appropriate method for test substance application to the seedlings, depending on the expected route of exposure of plants in the environment and the properties of the test substance - minimum 5 concentrations tested - minimum seed germination standards for the control (at least 70%) and control survival rate (at least 90%) - respecting test design and parameters (carbon dioxide level, humidity, photoperiods, temperature)
Results	EC10, EC50, NOEC and LOEC based upon survival IC10, IC50, NOEC and LOEC for each of roots, shoots and entire plant length IC10, IC50, NOEC and LOEC for each of roots, shoots and entire plant Weight
ISO 29200:2013 Soil quality - Assessment of genotoxic effects on higher plants - *Vicia faba* micronucleus test – the detection of micronuclei in the cells of secondary root tips of *Vicia faba*	
Time	2 stages – the development of primary roots (3days) - the development of secondary roots (4 days)
Endpoints	Micronuclei formation
Validity of the test	- using a negative and positive control - reference substance – maleic hydrazide - respecting test protocol
Results	Micronucleus frequency (number of micronuclei/1000 cells)

Triticum aestivum assay in environmental pollution monitoring

Environmental pollution is a very actual problem, and society must deal with it. The rapid industrialization and intensive agriculture continuously generate contaminants that affect the ecological balance and human health. In this context, efficient biomonitoring techniques are necessary to obtain an objective view of the matter. Environmental pollution monitoring using *Triticum aestivum* as test organism is mainly oriented in three directions: pesticides' soil pollution, heavy metals, and industrial waste pollution.

The toxic and adaptative responses of wheat plants to the investigated agents were evaluated using different parameters and biomarkers: germination, growth parameters, physiobiological parameters, enzyme activity (Table 2).

Germination and growth parameters

Seed germination is initiated by enzymatic reactions that activate catabolic and anabolic processes in the storage tissues and the embryonic axis. If these processes are disturbed by different xenobiotics, germination is inhibited. For example, copper exposure is associated with the decreased activity of amylase, an important enzyme that hydrolyzes the reserves of starch and releases the energy needed in the germination process. Therefore, the germination rate is often an investigated parameter in plant assays (Singh *et al*., 2007; An *et al*., 2009; Chen *et al*., 2010; Lamhamdi *et al*., 2011).

Growth inhibition is also a common outcome of exogenous pollutant toxicity, and retardation in growth potential and biomass accumulation are recommended parameters for predicting the toxicity of chemicals in plant assays (Zhang Q *et al*., 2016; Tripathi *et al*., 2017a).

Physiobiological parameters

Xenobiotic agents also induce changes at the biochemical level. The parameters that are most frequently evaluated are chlorophyll content, malondialdehyde level, and activity of antioxidative enzyme system.

The chlorophyll content is considered a sensitive indicator for monitoring damage to plant development. Superoxide anion free radicals level and lipid peroxidation are biomarkers used to evaluate oxidative damage in plant cells. Malondialdehyde is an oxidation byproduct of membrane lipids and it is regarded as an indicator of membrane lipids peroxidation and stress level (Zhang Q *et al*., 2016).

Plants possess a non-enzymatic antioxidative defense system, which includes carotenoids, phenolic compounds, and tocopherol. They act as reducing agents and their level increases under abiotic stress, leading to better adaptability under unfriendly conditions (Riaz *et al*., 2017). Proline is also an amino acid that accumulates in plants under environmental stress because it prevents membrane distortion and functions like a hydroxyl radical scavenger (Zhou *et al*., 2016).

Enzyme activity evaluation

The antioxidant defense is a basal physiological response in plants when they come in contact with xenobiotics. Plants have an endogenous antioxidant enzymatic system, designed to act as a defense system in case of induced abiotic oxidative stress (Fig. 2). Organic pollutants usually determine the excessive accumulation of reactive oxygen species in plants, triggering a rapid response, represented by modifications in enzymes' activity. The accumulation of O_2^- and H_2O_2 in plants exposed to pollutants determines the activity increase of the antioxidant enzymatic system (Li *et al*., 2008; Jiang *et al*., 2016; Zhang Q *et al*., 2016). As a response to the excessive production of O_2^-, the activity of SOD is intensified, as it catalyzes the transformation of O_2^- to H_2O_2 and O_2, protecting cells against oxidative damage. But abundant H_2O_2 is also nocive to plants, and its accumulation triggers an increase in the activity of enzymes like CAT and POD. However, in the late stages of oxidative stress, increased POD activity may lead to reactive oxygen species formation and lipid peroxidation. In some cases, very high levels of H_2O_2 may affect and reduce the activity of some enzymes. Glutathione S-transferases catalyze the formation of glutathione conjugates with xenobiotics, and their activity is stimulated as a response to pollutants aggression (Zhang *et al*., 2016).

However, the examination of antioxidant enzyme gene transcription is considered a more effective tool for assessing plant exposure to environmental stress than determining the antioxidant enzyme activity. In accordance, many studies also investigated this aspect, and demonstrated that xenobiotics can induce the expression of multiple genes, including genes relevant to antioxidation, and that plants can adjust their levels of enzymes through both molecular and physiological mechanisms to alleviate xenobiotics induced stress (Jiang *et al*., 2016; Wang and Zhang, 2017; Qu *et al*., 2019a; Qu *et al*., 2019b).

If the level of induced oxidative stress is too high, the capacity of the endogenous antioxidant system is overwhelmed, and the plants' structure is irreversibly damaged (Riaz *et al*., 2017).

Pesticides/Herbicides pollution

Agrochemicals are widely used in contemporary agriculture, but they accumulate in soil and in the water system and may exhibit toxic effects for plants or other living organisms. These chemicals interfere with the plants' physiological and biochemical processes, affecting their development. The accumulation of agrochemicals in soils and aquatic systems in high concentrations represents a key challenge in ecosystem health. Pesticide/herbicide residues can cause oxidative stress on crops, triggering an increase in the level of reactive oxygen species and in the activity of antioxidative enzymes. If the plants' adaptative antioxidative system is overwhelmed, signs of oxidative damage appear (lipid peroxidation). Another indicator for monitoring pesticide-induced stress to plants is the reduced chlorophyll content (Wang and Zhou, 2006b; Jiang *et al*., 2016).

Heavy metals pollution

Heavy metals contamination is a major environmental issue, and the level of contamination has increased parallel to industrialization. Bivalent metal cations (lead, mercury, cadmium, zinc, copper) are the most abundant. Zinc and

copper are essential micronutrients, but at high concentrations, they display toxic effects. Toxic levels of metals affect germination, plant growth and development, and all plant processes that regulate photosynthesis, water, and mineral nutrition status. Metals have the ability to interact with important functional groups in macromolecules and they modify the activity of several enzymatic systems. Furthermore, heavy metals induce overproduction of reactive oxygen species, causing oxidative stress and cellular damage. Seed germination is a very sensitive parameter to the impact of heavy metals and it is very often used to assess their phytotoxicity (Munzuroglu and Geckil, 2002; Lamhamdi *et al*., 2011).

In a comparative study conducted by Munzuroglu and Geckil (2002) on the effects of several metals on seed germination, root elongation and coleoptile and hypocotyl development in *Triticum aestivum* and *Cucumis sativus,* they concluded that mercury had the highest phytotoxic effect, with the highest germination inhibition percentage. Cooper and cadmium were also rated as unfavorable metals for plant growth

Plants have the ability to accumulate the toxic metals from the environment, and plants growing in metalliferous habitats probably develop tolerance mechanisms against metals. This adaptation poses an important threat to human beings when these plants are incorporated into the food chain (Munzuroglu and Geckil, 2002).

Industrial and domestic waste pollution

Rapid industrialization can have a negative impact on the ecological balance. Contaminants are produced in all industrial branches and they can be detected in sewage effluents, surface and ground waters, soils and sediments. On the other hand, a significant proportion of pollutants can originate from the household waste stream and from livestock operations. Combinations of pharmaceutical substances occur in the environment due to excessive prescription, consumption, and inadequate disposal. Efficient biomonitoring is essential. Plant test systems are more reliable and accurate than chemical assays, are easy to perform (plants can be directly grown on test samples, without extraction or pretreatment), and present good correlation with other biological assays (Bhat *et al*., 2019).

Triticum aestivum is a plant test model commonly used to assess the toxicity of industrial effluents/sludges. Various studies used *Triticum* assay to investigate the toxicity of several contaminates surfactants, pharmaceuticals, flame retardants, solvents, other organic pollutants (aniline, perfluoroalkyl derivatives). The main results are summarized in Table 3.

As presented above, ecotoxicological assessments represent a research subject of great interest. Several terrestrial plant tests are available to estimate the impact of different chemicals. Parameters frequently used as endpoints for phytotoxicity are seed germination, roots elongation, early seedling growth tests, photosynthesis inhibition and enzyme content fluctuation (Cao *et al*., 2007).

Table 2. Investigated parameters to assess the phytotoxic effects on *Triticum aestivum*

Germination and growth parameters	Germination percentage Elongation (roots and shoots) Biomass (dry/fresh weight)
Physiobiological parameters	Chlorophyll content Superoxide anion free radicals level Lipid peroxidation (malondialdehyde content) Soluble protein content
Enzymes activity	Superoxide dismutase (SOD) Catalase (CAT) Peroxidase (POD) Ascorbate peroxidase (APX) Glutathione reductase (GR) Glutathione – S- transferase (GST)

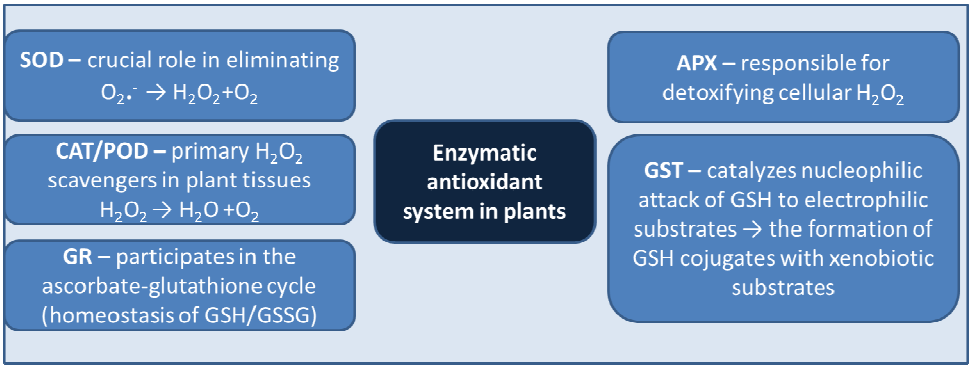

Fig. 2. The role of enzymatic antioxidant system in plants

Table 3. Environmental monitoring studies using the *Triticum* assay

Type of pollution		Observed effects	References
Pesticides/ Herbicides	Simetryne	• Suppressed growth • Decreased chlorophyll content • Signs of induced oxidative stress (over production of oxygen reactive species, membrane lipid peroxidation, activation of antioxidant enzymes)	Jiang *et al.*, 2016
	Imazethapyr	• Inhibitory effect on shoot weight and root weight (stronger for R enantiomer) • Oxidative damage (increased anthocyanin and malondialdehyde content, inhibited SOD and CAT activities	Qu *et al.*, 2019b
	Metolachlor	• Growth inhibition (stronger for S-enantiomer) • Oxidative stress (increased levels of superoxide anions and malondialdehyde, attenuated the expression of several antioxidant genes)	Qu *et al.*, 2019a
	Chlorotoluron	• General growth inhibition (roots, shoots and biomass) • Lipid peroxidation • Decreased chlorophyll content • Increased H_2O_2 accumulation • Activation of antioxidative enzymes	Song *et al.*, 2007
	Chlorpyrifos	• Growth inhibition • Reduced chlorophyll content • Disruption of the antioxidative enzymes system • Toxicity reduced by exogenous application of salicylic acid	Wang and Zhang, 2017
	Chlorimuron-ethyl	• Elevated malondialdehyde content • Reduction of the chlorophyll content • Increased activity of antioxidative enzymes (at first stage of exposure) • Completely lost defensive effect of antioxidative enzymes with prolonged exposure	Wang and Zhou, 2006a; Wang and Zhou, 2006b
Heavy metals / Toxic elements	Lead	• Inhibited germination • Negative impact on roots and shoots development • Induced catabolic metabolism disorders (reduced α-amylase activity and increased esterase activity) • Markers of lipid peroxidation and oxidative stress • Increased proline content – a non-specific defense reaction against lead toxicity	Lamhamdi *et al.*, 2011
	Mercury	• Inhibited germination • Inhibited roots and shoots elongation	Munzuroglu and Geckil, 2002
	Copper	• Inhibited germination • Inhibited roots and shoots development • Decreased amylase activity • Increased catalase and peroxidase activities	Munzuroglu and Geckil, 2002; Singh *et al.*, 2007
	Cadmium	• Growth inhibition for roots and shoots • Reduced total soluble protein levels • Genotoxic effects	Azimi *et al.*, 2013; Chen *et al.*, 2010
	Cobalt	• Moderate reduction of the germination rate • Inhibition of roots and hypocotyl development (complete above a concentration of 7.5 mM Co)	Munzuroglu and Geckil, 2002
	Arsenic	• Inhibition of roots elongation • High accumulation of reactive oxygen species • Increased activity of antioxidative enzymes • Alteration of mitotic process	Abbas *et al.*, 2018; Cao *et al.*, 2007
Industrial/ Domestic waste	Nonylphenol polyethoxylates	• Germination and growth inhibition (more intense for short-chain derivatives) • Markers of lipid peroxidation and oxidative stress	Zhang Q *et al*, 2016
	Polycyclic musks	• Germination inhibition (above 194 mg/kg) • Growth inhibition (more intense for shoots than for roots)	Chen *et al.*, 2010
	Tetrabromo-bisphenol A	• Lower chlorophyll content • Increased levels of malondialdehyde (lipid peroxidation) • Induced oxidative stress – increased activity of SOD, POD and CAT (first stage of stress) • Decreased activity of POD and CAT (prolonged exposure)	Li *et al.*, 2008
	Aniline	• Suppressed plants development (shoot/root height, dry/fresh weight) • Genotoxic effects (decreased mitotic index and increased frequency of micronuclei and chromosomal aberrations)	Tao *et al.*, 2017
	Perfluorooctanoic acid	• Inhibited germination (dose-dependent manner) • Affected roots and shoots growth (increased at doses < 0.2 mg Kg^{-1} / decreased at doses > 800 mg Kg^{-1}) • Increased proline content • Increased POD activity and inhibited CAT activity	Zhou *et al.*, 2016

Pharmaceutical compounds	Perfluorooctane sulfate	Below 10 mgL^{-1}: ● Slight stimulation of seedlings growth ● Slight stimulation of chlorophyll and soluble protein synthesis ● Enhanced activities of SOD and POD Above 10 mgL^{-1}: ● Inhibition of elongation and biomass reduction ● Inhibition of chlorophyll and soluble protein synthesis ● Inhibition of SOD and POD (damage of the antioxidative defensive system) ● Increased electrolyte leakage	Qu *et al*., 2010
	Imidazolium-based ionic liquids	● Inhibitory effects on seedlings growth ● Decreased pigment (carotenoid and chlorophyll) content ● Induced oxidative stress	Xu *et al*., 2018
	Quaternary ammonium compounds	Concentrations > 0.8 mg L-1 ● Inhibition of plant growth ● Reduction of photosynthetic pigment content ● Oxidative stress and membrane lipid peroxidation	Li Y *et al*., 2019
	Paracetamol	● No significant influence on germination frequency ● Inhibition of roots and shoots elongation (greater for roots) ● Chlorophyll content reduction and macroscopic symptoms of treated wheat leaves ● Lower soluble protein content ● Modified activity of POD and SOD enzymes, as a typical stress response	An *et al*., 2009
	Fluoroquinolone	● No influence on germination ● Significantly reduced growth parameters ● Cellular membranes damage – increased MDA content ● Evidence of oxidative stress (increased total phenolic content, antioxidative enzymes' modified activity)	Riaz *et al*., 2017
	Sulfadiazine	● Inhibition of shoots and roots elongation ● Inhibition of the chlorophyll synthesis rate ● Increased activities of SOD, POD and CAT ● High lipid peroxidation and membrane damage	Jin *et al*, 2009; Xu *et al*., 2017

Genotoxicity assessment using *Triticum aestivum* assay

Toxicology guidelines promote the substitution of assays using animal models with other bioassays. In this sense, genotoxicity assays using higher plants models have gained in popularity. However, the issues of reliability of plant chromosome aberration bioassays and the possibility of results extrapolation to humans are still questioned by some scientists. Comparison studies between comet assay using plant root cells and human leukocytes demonstrated a correlation between results obtained for plant cells and human cells, proving that DNA damage is universal, independently of the physiology of the organism itself (Reis *et al*., 2017).

Besides their utility in risk assessment in ecotoxicology, plant bioassays have many other applications (Fig. 3). They may represent an alternative to reduce the number of animals used in anticancer research. Olaru *et al*. (2019) conducted a study to validate the *Triticum aestivum* root elongation assay as a simple and efficient alternative tool for the assessment of the potential of novel anti-proliferative agents. They tested 20 anti-proliferative compounds in the *Triticum aestivum* root elongation assay and compared the results with the NCI60 human tumor cell line anti-proliferative profile. The results of the study pointed out that the *Triticum* test used alone has significant limitations, due to false-negative results, but in combination with other bioassays can be a useful tool to detect novel anti-proliferative agents, particularly those targeting tubulin (Olaru *et al*., 2019).

Another area where plant bioassays can be successfully used is genotoxicity assessment of medicinal plants and extracts. Due to the extensive use of medicinal plants, it is very important to evaluate the safety and the genotoxic and mutagenic potential of herbal medicines during the preclinical evaluation (Sponchiado *et al*., 2016; Trifan *et al*., 2012; Trifan *et al*., 2013).

Triticum aestivum is one of the plant species frequently used in genotoxicity assessment (Table 4). The selection of wheat as plant model for genotoxicity evaluation is favored by the fact that the *Triticum* chromosomes are large and appropriate for micronucleus and chromosome abnormality assays (Tao *et al*., 2017).

One of the most common investigated parameters is the mitotic index (the ratio between the number of cells in division and the total number of evaluated cells). There are several mechanisms that can explain the mitodepressive effect induced by different chemical agents. The inhibition in mitotic activity can be related to the inhibition of DNA-polymerase, necessary for the synthesis of DNA precursors or to the disruptions that appeared in the biosynthesis process of some cell cycle key proteins directly involved in spindle assembly or orientation. Oxidative stress also represents a key factor, one that mediates genotoxicity indirectly. Reactive oxygen species cause various lesions to lipids, proteins, and DNA, which lead to important cellular dysfunctions (Azimi *et al*., 2013; Truta *et al*., 2013).

Heavy metals can cause genotoxicity due to the direct or indirect (through hydrogen bonding) interaction of the metal with the DNA molecule. The interactions of metals with DNA bases usually disrupt base-pair hydrogen bonding and destabilize the double helix. Cd acts as a mutagen primarily by direct inhibition of an essential DNA mismatch repair, resulting in a high level of genetic instability (Azimi *et al*., 2013).

Several chromosome aberrations were identified. Aneuploidy (an abnormal number of chromosomes in a cell) can be associated with the induction of lagging chromosomes, that lost their ability to attach by spindle fibers and do not participate in the normal division (Truta *et al*., 2013).

The appearance of bridges, fragments, and micronuclei is determined by the DNA damage caused by the tested agents. Cell death is further associated with unrepaired damage to the DNA molecule in exposed cells (Silveira *et al*., 2017).

Table 4. Genotoxicity studies using *Triticum aestivum* assay

Tested agent	Methods used	Observed effects	Reference
Copper salts (copper acetate monohydrate, copper citrate)	Cytogenetic analysis	• Reduced mitotic index • Reduced prophase index • Increased metaphase and ana-telophase index • Increased percentage of ana-telophase chromosome aberrations (chromosome bridges, laggards, complex aberrations)	Truta *et al*., 2013
Cadmium	Random amplified polymorphic DNA	• Disappearance or appearance and alteration in intensity of bands	Azimi *et al*., 2013
Boric acid and borax	Comet assay	• DNA damage	Sahin *et al*., 2012
Aniline	Cytogenetic analysis	• Reduced mitotic index (severe inhibition at concentrations > 10 mg L^{-1}) • Increased frequency of micronuclei • Increased frequency of chromosomal aberrations (chromosomal bridges, chromosomal fragments, chromosome multi-stage split, interphase nucleus leakage)	Tao *et al*., 2017
Cinnamic acid derivatives	Cytogenetic analysis	• Inhibited the mitotic activity • Presence of ana-telophase • 4 types of chromosomal aberrations: chromosome bridges and fragments, micronuclei, and multipolar ana-telophases	Jitareanu *et al*., 2013
Tetracycline	Cytogenetic analysis	• Concentrations < 1mg L^{-1} –stimulated mitotic division • Concentrations > 50 mg L^{-1} - reduced mitotic index • Increase in the frequency of micronuclei, chromosomal aberration, and sister chromatid exchange	Xie *et al*., 2011
Graphene oxide	Cytogenetic analysis	• Increased mitotic index – due to high number of prophases • Presence of chromosome aberrations - clastogenic/aneugenic effect	Vochita *et al*., 2019
TiO2-NP	• Analysis of ploidy stability • Comet assay • Micronucleus test	• Similar genome size for control and treated plants • DNA damage • Increased number of micro-nucleated cells	Silva *et al*., 2017
Ag-NP	Cytogenetic analysis	• Extensive damage caused to DNA • Inhibition of DNA synthesis during the S-phase of interphase • Decreased mitotic index Induction of chromosomal aberrations (incorrect orientation at metaphase, chromosomal breakage, metaphasic plate distortion, spindle dysfunction, stickiness, fragmentation, scattering, unequal separation, scattering, chromosomal gaps, multipolar anaphase, erosion, lagging chromosomes)	Abdelsalam *et al*., 2018

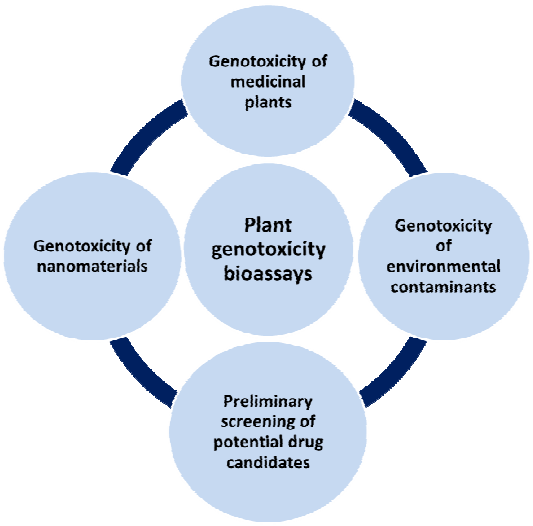

Fig. 3. Applications of genotoxicity plant bioassays

Phytotoxicity evaluation of nanomaterials using *Triticum aestivum* assay

Nanotechnology has a wide range of applications, from medicine, pharmaceutic industry (targeted drug delivery) to electronics and biosensors. The field of nanotechnology is a very dynamic scientific domain, with important applications especially in nanomedicine and nanopharmacology, where engineered nanoparticles were developed as smart drug-delivery systems. In this context, nanotoxicology investigations have also become a research field of interest.

The investigation of different nanoparticles in plant assays has two major purposes. The first one is to find applications of nanotechnology in the agriculture sector (Vicas *et al*., 2019), as nano-fertilizers, new tools to deliver particular bioactive molecules to manipulate plant breeding and genetic transformation, new approaches for intracellular labeling and imaging, and potential agents with beneficial effects under adverse environmental conditions - eg. heavy metal stress. The second perspective focuses on evaluating the toxic potential of these entities in plants and their impact on the environment, and ultimately to human health (Wang *et al*., 2016; Rizwan *et al*., 2019).

The most important physico-chemical feature of nanoparticles is their increased surface/volume ratio, which enhances their reactivity and their capacity to interact with roots exudates and specific membrane transporters (Giorgetti, 2018). Several studies demonstrated that nanoparticles can pass through plant cell wall pores, accumulating in all tissues, including newly developed seeds. Under these conditions, nanoparticles were also detected in the second generation plantlets (Larue *et al*., 2012). Three mechanisms were proposed to explain the penetration of nanoparticles into cells: direct diffusion through the lipid bilayer of cell membranes, endocytosis, and using ion channels and membrane transporters (Giorgetti, 2018).

Toxicity of nanoparticles to plants was observed at different levels: morpho-anatomical, physiological, biochemical, and genetic. Nanoparticles and plant interactions can result in reduced seeds germination rates, decreased photosynthesis, histological alterations, changes in the generation of secondary metabolites, and a negative influence on plant genome system (Tripathi *et al*., 2017a). Several plant bioassays investigated the toxicity and genotoxicity of nanoparticles, emphasizing the fact that cytogenetic analysis of root meristems represents the simplest genotoxicity studies, determining the mitotic index, chromosome breakages and anomalies, and micronuclei (Tripathi *et al*., 2017a; Giorgetti, 2018).

One mechanism proposed to explain the negative effects is the high production rate of reactive oxygen species, which leads to oxidative stress. The excess of reactive oxygen species promotes lipid peroxidation, affecting membranes' fluidity and permeability, and, in consequence, the acquisition of water and nutrients. It ultimately leads to retardation in growth potential. Oxidative stress is also associated with DNA damage (Tripathi *et al*., 2017a).

Other possible explanations could be dissolution of toxic metal ions, as Ag^{+}, Cu2+, blockage of plant nutrient transport channels caused by nanoparticles aggregation, binding interactions that release surface free energy, leading to surface reconstruction of biomolecular structures, and oxidation of biomolecules through catalytic reactions (Wang *et al*., 2016; Du *et al*., 2019).

Triticum aestivum is one of the plant species commonly used in many assays involving nanomaterials. Different types of nanomaterials were investigated; the results of the studies are presented in Table 5.

Metals and metal-oxide nanoparticles are a very important group of nanoparticles, with broad applications.

TiO_2 nanoparticles are among the most used nanoparticles, having multiple applications in many fields, including agriculture and pharmaceutical industry (Rafique *et al*., 2014). Titanium dioxide nanoparticles' interaction with plants represents a subject of interest, allowing ecotoxicological assessment, but also the evaluation of potential applications in agriculture, allowing for the controlled release of agrochemicals (e.g., fertilizers, pesticides, and herbicides) and target-specific delivery of biomolecules (Wang *et al*., 2016; Silva *et al*., 2019).

From a toxicological perspective, particle size is extremely important, influencing the interfacial reactivity and the ability to traverse physiological barriers. This fact was also confirmed in experiments performed on *Triticum aestivum*, Larue *et al.* observing size-dependent accumulation and translocation of TiO_2 nanoparticles, with only NPs<140 nm accumulated in roots and NPs<36 nm translocated into leaves (Wang *et al*., 2016).

Studies performed on *Triticum aestivum* with TiO_2-NP revealed that the biological effects connected closely to the concentration of exposure, a concentration below 60 mg kg^{-1} stimulating the development of both roots and shoots, while above this value, negative effects appeared. One possible explanation could be the enlargement of root pores at low concentrations of TiO_2-NP and, in consequence, the improvement of water and nutrients absorption, while high concentrations can cause root pores clogging, reducing water and nutrients supply (Rafique *et al*., 2014; Rafique *et al*., 2018b).

ZnO-nanoparticles can represent an alternative to classical Zn salts, like $ZnSO_4$, as fertilizers and a potential way to increase Zn content in crops and alleviate human Zn diet deficiency. Zn is an essential micronutrient implicated in many physiological processes, with high importance for both plant and animal organisms. Zn deficiency can have negative repercussions, affecting the synthesis of many enzymes, the biosynthesis of chlorophylls in plants, and also the immune response in animals and humans. Therefore, assuring an optimal level of Zn is very important.

Du *et al.* (2019) investigated the impact of ZnO NPs and $ZnSO_4$ on wheat, the results indicating that ZnSO4 was more toxic than ZnO NPs at high doses. The investigated parameters were seed germination inhibition, reduction of root and shoot length and dry biomass of seedlings. There was an indication of oxidant stress induction due to the overproduction of reactive oxygen species for both ZnO NPs and $ZnSO_4$, but the variation in enzyme activities was greater for ZnSO4. The study concluded that toxic responses were generally lower for ZnO NPs and that no ZnO NPs where found in any plant organs, no nano-risk being identified.

Table 5. Nanomaterials phytotoxicity studies conducted on *Triticum aestivum*

Tested materials	Observed effects	References
TiO_2-NP	• Germination rate similar to the controls • Growth stimulation in seedlings • Chronic exposure → reduced total length and biomass, chlorotic aspect • Oxidative stress – increased in leaves, decreased in roots • DNA damages, clastogenic effect	Silva *et al.*, 2017
	• Impairment of both light-dependent and -independent phases of photosynthesis • Reduction in chlorophyll a content, net photosynthetic rate, transpiration rate, stomatal conductance, intercellular CO_2 concentration • Reduced carbohydrates reserves • No effect on total soluble sugar content or in RuBisCO activity	Dias *et al.*, 2019
	• Shoot (not root) growth reduction • Organ dependent antioxidant responses • Higher capacity of roots to deal with induced oxidative stress in comparison with leaves	Silva *et al.*, 2019
ZnO-NP	• Concentration dependent responses • Up to 60 mg kg^{-1} – increased root and shoot lengths, P uptake, and chlorophyll content • 100 mg kg^{-1} – affected plant development and increased H_2O_2 production and micronuclei formation	Rafique *et al.*, 2018b
	• Increased root and shoot length and total fresh and dry biomass up to 60 mg/kg • Inhibitory effects and cell damage at concentrations higher than 60 mg/kg	Rafique *et al.*, 2014 Rafique *et al.*, 2018a
	• No significant change in contents of all amino acids and the total protein in wheat grains	Wang *et al.*, 2019
	• No significant inhibitions of the final germination rate for all ZnO NPs doses • Significant reduction compared with control for the root growth and shoot elongation at doses over 50 mg L^{-1} and 100 mg L^{-1} • Changes in root morphology (root hair and first-order and second-order lateral roots number) • All plant organs showed increased Zn content, but no ZnO-NP were found in plant tissues	Du *et al.*, 2019
	• Reduced Cd level in exposed plants • Decreased toxicity in Cd exposed plants - reduced the electrolyte leakage and superoxide dismutase and peroxidase activities • Positive impact on photosynthetic parameters (eg. chlorophyll a, chlorophyll b, carotenoid) • Increased Zn concentrations in plants	Hussain *et* al., 2018; Rizwan *et al.*, 2019
NiO-NP	• Reduced biomass production • Significantly decreased all photosynthesis related parameters, except for the content of carotenoids • Increased the endogenous Ni levels in plants • Oxidative stress and damage (at high phytotoxic levels)	Saleh *et al.*, 2019
Fe-NP	• Stimulated the growth of wheat plants exposed to Cd • Reduced the Cd uptake by wheat plants • Increased photosynthetic activity • Increased the Fe concentrations in plants – a possible way to the bio-fortification of cereals	Hussain *et* al., 2019 Rizwan *et al.*, 2019
Fe_3O_4-NP	• No negative effect on germination rate, the chlorophyll content, and plant growth • No biochemical signs of oxidative stress • No evidence of cell death estimated by Evans blue staining • No evident phytotoxic effects	Iannone *et al.*, 2016
CuO-NP	• Altered the level of essential amino acids in wheat grains (at high doses) - decreased the levels of threonine, histidine, isoleucine, leucine	Wang *et al.*, 2019
Cu-NP	• Reduced growth rate of roots, accompanied by stimulation of lateral roots formation • Oxidative stress and cell membrane damage, but less intense than for Cu ions	Zhang *et al.,* 2018
Ag-NP	• Genotoxic effects	Abdelsalam *et al.*, 2018
	• Toxic effect on the early growth of wheat seedlings, more evident on roots • Altered expression of several proteins mainly involved in primary metabolism and cell defense	Vannini *et al.*, 2014
Graphene oxide	• Induced oxidative stress • Clastogenic/aneugenic effect	Vochita *et al.*, 2019
Graphene	• Plant growth and photosynthesis inhibition at long time exposure (30 days) • Induced oxidative stress and nutritional disorder.	Zhang P *et al.*, 2016
Carbon nanotubes	• No evident toxicity for the germination process • Enhanced root elongation • No alteration in plant development or root-tissue morphology	Miralles *et al.*, 2012
Chitosan-NP	• Promoted the growth of wheat seedlings (<100μg/mL) • Improved the germination index, vitality index and biomass (<100μg/mL) • Improved the photosynthetic capacity and the total soluble protein content • Up-regulated indole-3-acetic acid synthetic genes	Li R *et al.*, 2019
Silicon-NP	• Reduced Cd toxicity in wheat plants • Enhanced the biomass and growth parameters of Cd-stressed wheat • Improved mineral uptake and increased the photosynthesis • Reduced oxidative stress markers • Reduced the Cd concentration and its translocation towards aerial parts and grains of wheat	Ali *et al.*, 2019
	• Reduced UV-B stress • Photosynthesis and oxidative stress regulation	Tripathi *et al.*, 2017b

Rizwan *et al*. (2019) analyzed the influence of zinc and iron oxide nanoparticles on the development of wheat plants under metal (cadmium) stress. Both types of NPs increased plants' biomass, nutrients uptake and decreased Cd toxicity biomarkers. Furthermore, the concentrations of Zn and Fe significantly increased upon exposure to ZnO and Fe_2O_3 NPs, accompanied simultaneously by the reduction of Cd contents, a toxic heavy metal. The positive influence of ZnO-NP in reducing the toxicity and concentration of Cd in wheat was also reported by Hussain *et al*. (2018).

Magnetite iron oxide nanoparticles presented no phytotoxic effect on wheat (at concentrations of 5, 10, 15 or 20 mg L^{-1}). Fe_3O_4-NP treatment did not affect seed germination or plant growth. The chlorophyll content was similar to the control and no signs of oxidative stress were identified (lipid peroxidation and membrane damage, or reactive oxygen species accumulation). Furthermore, the activity of the enzymatic antioxidant defense system was superior to the control, preventing potential oxidative damage in wheat tissues (Iannone *et al*., 2016).

Iron nanoparticles had a positive influence on wheat under Cd stress. Fe-NP application enhanced wheat growth, the chlorophyll a, b and carotenoids concentrations, and the activity of antioxidant enzymes in plants exposed to toxic levels of cadmium. These beneficial effects were also associated with the reduction of Cd uptake by plants (Hussain *et* al., 2019; Rizwan *et al*., 2019).

Silver nanoparticles are widely used in medicine, mainly due to their antimicrobial potential. Their toxic effects are not limited to bacteria and fungi, but also affect other organisms, like plants and mammals. Several toxicity mechanisms were proposed by researchers for Ag-NP. They can be summarized to oxidative stress, DNA damage, lipid peroxidation, membrane, and mitochondrial damage, the release of Ag+ in solution, and the ion-exchange hindering and exocytosis disrupting processes (Du *et al*., 2018).

Many different types of aberrations were identified on *Triticum aestivum* root tip cells- c-metaphase, fragments, bridges, uncoiling, stickiness, deletions, distributed metaphase and anaphase, multiple nuclei, elongation, cap chromosomes, eroded and lagging chromosomes, ring chromosomes and multipolar anaphase (Abdelsalam *et al*., 2018).

Vannini *et al*. (2014) also exposed wheat plants to Ag-NP and concluded that roots were more susceptible to the toxic effects of silver nanoparticles and that toxicity was primarily related to the Ag ions released by oxidative dissolution of NPs at the root interface.

Jośko *et al*. (2017) investigated the phytotoxic effect (seed germination and root growth) of several mixtures of nanoparticles (ZnO-NP, CuO-NP, Cr_2O_3-NP, TiO_2-NP, Fe_2O_3-NP) on four plant species, *Triticum aestivum* being among them. Interestingly, the toxic effects produced by the combined mixtures of NPs were lower than those exhibited by individual mixtures. One possible explanation could be the greater aggregation of nanoparticles in combined stress conditions; larger aggregates present a lower specific surface area and a lower degree of solubility (Jośko *et al*., 2017).

Carbon-based nanomaterials, such as fullerene, carbon nanotubes, and graphene, have many applications in engineering and medical fields.

Graphene and graphene oxide belong to a class of carbon nanomaterials, in the form of two-dimensional sp2-hybridized sheets. These materials are very important in the fields of nanoelectronics, biomedicine, energy storage devices, and adsorption materials. The environmental impact of graphene oxide was studied using different plant species, *Triticum aestivum* being one of them.

Zhang *et al*. (2016) observed that exposure enhanced the elongation of wheat roots, but concluded that the overall effect was not a positive one. Graphene affected the development of root hairs and there was also evidence of induced oxidative stress. Long-term graphene exposure induced physiological, metabolic changes in plants, affecting the plant's nutritional status and biomass production and also causing visible alterations in shoots (Zhang *et al*., 2016). Another study revealed the clastogenic/aneugenic effect of these carbon nanomaterials. Several aberrations were identified - bridges (simple and multiples), fragments and micronuclei, all pointing to the clastogenic effect of GO, especially at high concentration (Vochita *et al*., 2019).

Multiwalled carbon nanotubes' phytotoxicity was also evaluated on *Triticum aestivum*. The plants tolerated high concentrations of industrial-grade carbon nanotubes and even exhibited enhanced root development and germination in their presence (Miralles *et al*., 2012).

Polymer-based nanomaterials present high biological safety and good biodegradability and are used especially in the field of drug development, but also in agriculture, to improve growth and reduce abiotic stress in crop plants.

Various methods to alleviate environmental stress in plants were studied. Exogenous supplementation of silicon proved to be one of the most feasible techniques. Silicon nanoparticles were efficient in reducing toxicity associated with Cd exposure. Cd stress caused oxidative stress and impairment in plant nutrients, affecting the growth and development of plants. The supplementation of silicon nanoparticles significantly reduced the Cd concentration in *Triticum aestivum* plant tissues, ameliorated photosynthetic parameters, and reduced oxidative stress biomarkers. It reduced electrolyte leakage and enhanced the activities of superoxide dismutase and peroxidase in leaves (Ali *et al*., 2019).

Silicon nanoparticles also proved to be effective than silicon in alleviating UV-B stress in wheat (*Triticum aestivum*) seedlings. UV-B stress in wheat plants manifested through a negative impact on seedlings' growth declined photosynthetic performance, increased levels of superoxide radical and H_2O_2, and enhanced lipid peroxidation and electrolyte leakage (Tripathi *et al*., 2017b).

Chitosan nanoparticles had a positive influence on the germination and seedling growth of wheat at low dosage, but Behboudi *et al*. (2017) pointed out that high concentrations can have toxic effects, probably explained by the induced aggregation of the particles that resulted in clogging of root pores, which interrupted water uptake by seeds (Behboudi *et al*., 2017).

According to the data presented above, *Triticum aestivum* is a plant species that is suitable for phytotoxicity evaluation of nanoparticles. Nanoparticles exhibited both negative and positive effects on plants, and their study

represents an area of great interest, due to their potential applications in agriculture, but also to their impact on the food chain and environmental sustainability. The toxic effects of nanoparticles are connected to their characteristics (size, dissolution capacity, concentration), but also depend on the plant species (Zia-ur-Rehman *et al*., 2018). Their phytotoxic potential is mainly materialized in altered stomatal conductivity and decreased photosynthesis, alteration in the acquisition of water and nutrients, impediment in the generation of secondary metabolites, and influence on the genome system.

Conclusions

Plant assays are a reliable tool for toxicity and genotoxicity assessment. *Triticum aestivum* is one of the recommended test species by international guideline. In the context of toxicology guidelines that promote the substitution of assays using animal models with other bioassays, genotoxicity assays using higher plants models have gained in popularity. Although there are studies that demonstrated that the results obtained for plant cells were in agreement with the effects observed on human cells, the mutagenesis and carcinogenesis potency of different agents cannot be extrapolated directly to humans. However, these techniques that use vegetal cells to investigate genotoxicity can provide some important pieces of information. At present, plant assays are extremely important for the risk assessment of environmental pollutants, due to their simplicity, reliability, and low cost, but their applications can extend beyond the boundaries of environmental monitoring.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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