International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.2, pp 961-967, 2017

AReview on *Vicia faba* as a plant test system in toxicity evaluation of various metals: Vicia-micronucleus test (Vicia-MCN)

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Abstract : Toxicity evaluation is a vital practice for evaluating various toxic levels excreted by pollutants. The pollutants were harmful to the environment and it may exhibit serious hazards to the mankind. We need tools to rule out the toxicity levels of pollutants in short period of time. In this regard, Vicia-micronucleus test was a plant test system widely used for evaluating toxicity in various applications. *Vicia faba* plant system is a reliable, easy, sensitive and efficient biological system to carry on genotoxicity and other related studies. Majority of the countries worldwide grow *Vicia faba*, a legume, which has ability to grow in any environmental conditions. However, the damage caused by pollutants to this plant can be treated using breeding analysis. This review article focuses on *Vicia faba's* role in toxicological studies as a test plant system and breeding analysis.

Keywords : *Vicia faba*; Legume; Vicia-micronucleus test; Toxicity; Pollutants.

Introduction

Since many years, due to industrialization, our environment is polluted by various pollutants like metals [1, 2, 3]. Currently, various chemical analysis are available to determine toxic levels, however, reliable biological tests are required to assess the exact damage caused by pollutants[4]. A well know *Vicia*-micronucleus assays (Vicia-MCN) have been used to detect the genotoxic effects of various pollutants, metals, radioactive materials. This simpler method was cheap, easy and widely used in many studies to identify oxidative damage, genotoxic effects and mitotic index (MI) [5]. The *Vicia faba* (*V. faba*) is common plant grown in major parts of the world. It is a legume, with high nutrition belongs genes *Vicia* and family Fabaceae[6, 7]. It is also known as Broad bean, Horse bean, Windsor bean, Tick bean, Fava bean. Despite of its origin in the east,the plant was grown all over the world as it is favorable in all climatic conditions [8- 11]. Apart from genotoxicity evaluation the *V. faba* is used to evaluate the efficacy of herbicides like glyphosate and Imazapic against *Orobanche crenate* [12]. The foliar evaluation was done using *V. faba* plantas a model[13-15]. Similarly, Gibberellic acid, Uniconazole, Cobalt, α-tochopherol, Citric acid, Oxalic acid effect of growth was tested using *V. faba* plantas a model[16-19].

Genotoxicity can be defined as toxicity to the genome [20]. The legume, *V.faba* plant was widely employed for micronucleus assay by which genotoxicity in various applications can be determined. The test was performed by exposing the *V.faba* plant to pollutants in various concentrations. Further, the plant or plant parts were analyzed for genotoxicity evaluation[21]**.** This test is standardized by French Member Organization as a prominent test to determine the toxicity [4]. The toxicity executed by these pollutants can be alleviated by new breeding techniques. Some of the important genotoxicity evaluation using *V.faba* asplant system and breeding analysis is briefly described in this review.

Cesium genotoxicity evaluation

Vicia-MCN plant bioassay is used to study the genotoxicity of radioactive materials. ^{137}Cs is harmful to human beings. DNA damage by ^{137}Cs (Radioactive) is assessed by Vicia-MCN and other test methods like tradescantia-micronucleus test (Trad-MCN). In addition, Tradescantia-Stemen-hair mutation test also carried out to show toxic effects of ^{137}Cs. In vicia micronucles test (Vicia-MCN) the *V. faba* seeds were soaked in ionized water and it is allowed to germinate at a temperature of 22 degrees centigrade for three days and it is allowed to grow under nourishment of hoagland's solution for four days. This test results showed genotoxicity of ^{137}Cs, concluding this method is a sensitive and cost effective bio assay in detecting genotoxicity of ^{137}Cs. Likewise, this bioassay can be used to evaluate genetoxicity of various radioactive materials which is contaminated in soil and water [22]**.**

Sulcotrione genotoxicity

Sulcotrione (2-(2-chloro-4-(methylsulfonyl) benzoyl)-1, 3 cyclohexanedione), is an herbicide used to control grasses weed. The genotoxicity of sulcotrione was determined using Vicia-MCN as treatment system. In brief, the *V.faba* seedlings is treated for 45 h with sulcotrione, after treatment for 42h, potent mutagenic effect in hydroponic conditions was observed[23]. The study results prove Sulcotrione cause genotoxicity in plants.

Antimutagenic activity of *origanum majorana*

Origanum majorana (O. majorana) is awidely used medicinal plant in folk medicine, Saudi Arabia.Using Vicia-MCN as a control test system the antimutagenic activity*O. majorana* was evaluated.In this study, the root tips of *V.faba* and *O. majorana* are treated with Sodium azide in concentrations of 250 and 350µg/ml and 50, 100, 200µg/ml for 6 h and 20 h, respectively. The study results show no aberrations in sodium azide treated root tips of*O. majorana*with the control, where no aberrations found. This study is an evidence that *O.majorana* has anti-mutagenic activity using *V.faba* plant system as control [24]**.**

Arsenic (As) genotoxicity evaluation

Arsenic toxicity through water has been reported in many parts of the world. Arsenic is an element, which is highly toxic to humans. Arsenic toxicity can be evaluated using simple, efficient and reproducible bioassay, Vicia-MCN.It causes skin, bladder, lung, liver cancers, and also serious effects like neurotoxicity, birth defects, and metabolic disorders. When arsenic is given to plant models i.e., Vicia-MCN,modifications in the root color, stiffened roots, and decrease in root length, mitotic index and chromosomic aberrations was observed. In addition, arsenic can cause oxidative stress, cytotoxicity, and genotoxicity in plants [5, 25].

Cadmium (Cd) genotoxicity evaluation

Cadmium is a metal, which is present in air, water and soil. Cadmium can induce genotoxity and toxic effects by inducing micronuclei in the root tips of *Vicia* species. Vicia-MCN was carried out in soil spiked with $CdCl_2$ at different concentrations. Experiment was carried out in two terms, short term and long term. In the short term *V.faba* seeds were exposed to soil spiked with $CdCl_2$ at different concentration for a period of five days. Similar treatment was given in long term experiment for a month. Genotoxicity was observed in both short and long term experiments. This study is an indication that micronucleus assay can be used as a marker for determining the genotoxicity of plants. Similar results were found, when *V. faba* is treated with low concentrations of cadmium,which caused genotoxicity and micronuclei was found after 48th hour[21,26].

Copper (Cu) genotoxicity evaluation

Copper (Cu) is an essential element for nutrition of humans as well as for plant growth. The toxicity of Cu was determined using Vicia-MCN. Interestingly, *V. faba* was found to be resistance to the toxic effects of Cu. In a study,*V. faba* is collected from heavy metal contaminated soil to evaluate its activity under Cu stress. The improvement of biomass and faba bean resistance to the toxic effects of Cu was noted. Whereas, low levels of Cu shown beneficial effects in *V.faba* plant by promoting growth of the plant. Additionally, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase

(POX) was found in high concentrations. Toxicity was observed when the plant was exposed to copper level more than >2mM concentration[27]. It is important to note the levels of Cu present in soil as it is beneficial to plants in upto a certain concentration (>2mM).

Lanthanum (La) genotoxicity evaluation

Lanthanum (La) is a rare earth metal, can react with nucleoproteins, amino acids, phospholipids, enzymes, intermediate metabolite. In china, La is used in fertilizers for high yield crop production. However, when La was studied using Vicia-MCN test system, reactive oxygen species (ROS), HSP70, Isoenzymes like endopeptidase, superoxide dismutase (SOD), catalase (CA), ascorbate peroxidase (APX), guaiacolperioxidase (GPX) are produced in order to alleviate La-induced oxidative damage. The production of ROS and other enzymes is an indication that La is toxic to plants as well to animals. The toxicity levels of La could be determined by quantitative measurement of these enzymes [28].

Lead (Pb) genotoxicity evaluation

Toxicity of lead (Pb) is evaluated using Vicia-MCN. Pb is widely used metal in various applications, but it exerts toxicity to animals and plants. It inhibits plant growth, also causes medical complications to human health and believed to be a possible carcinogen. Pb induced DNA fragmentation was observed at peak levels at 10 M concentration and thereafter the gradual decrease of toxicity was noted. So we can determine Pb was toxic to plants at threshold concentration [29].

Aluminium (Al) genotoxicity evaluation

Aluminium (Al) is a metal, which is toxic to plants by inhibiting the growth of roots. The investigation of Al toxicity using Vicia-MCNand *Zea mays* as plant system showed Al was toxic in both dicot and monocot plants. Using Laser Microprobe Mass Analysis (LAMMA) localization of Al was assessed in root tips. Al has reached stele in *Zea mays* and it is confined to outer cortex in*V.faba*. This indicates that Al has stronger affinity in dicots. However, Al was seen intracellular in 60 mins of treatment in both dicots and monocots. So, it is well understood, long term exposureto Al can induce toxicity in both monocots and dicots [30].

Selenium (Se) genotoxicity evaluation

Vicia-MCN and sister chromatid exchange (SCE) tests were employed to evaluate selenium toxicity. Selenium is important and essential trace element with anti-mutagenic and anti-cancer properties. Its deficiency may cause certain types of cancers and cardiovascular diseases. But it is conceived to be toxic at higher concentrations and it is toxic to humans and animals. The evaluation of sodium selenite and sodium biselenite showed an increase in MN frequency, SCE frequency and decrease in mitotic index (MI) indicating selenium exhibits genotoxicity [31].

Sulfur dioxide (SO_2) genotoxicity evaluation

Yi and meng in 2003 investigated genotoxicity of sulfur dioxide (SO_2), a common air pollutant. SO_2 and its hydrates, bisulfite are investigated using *Allium stavium* and Vicia-MCN bio assay, considering it as simple, and efficient bio assay. In this study, both the plants are treated with the mixture of SO_2hydrates at different concentrations. The results of this study showed SO_2exhibits genotoxicity, increase in Micronuclei (MN) frequencies was observed [32].

Parthenin genotoxicity evaluation

Allelotoxic effects of parthenin, a natural constituent of *Parthenium hysterophorous*. *Parthenium hysterophorous* is a highly spreading weed in India and other parts of the world was studied using Vicia-MCN. In brief, the seeds of *V.faba* are treated with parthenin for 8 h in concentrations of 100,200,300 and 400 IM. It has been observed that high concentration of parthenin exhibits mutagenic and cytotoxic which affects the growth, quality and quantity of the plant, when compared with control [33].

Municipal solid waste (MSW) landfills toxicity evaluation

Worldwide, municipal solid waste (MSW) landfills are widely employed to dispose the waste. In china, landfill leachate was collected from different seasons and evaluated using Vicia-MCN. The MSW samples has exhibited decrease in the mitotic index (MI) and increase in Micronucleus(MN) in *V.faba* root tips was observed. This results provide an evidence MSW could be harmful to plants and humans[34].

Silver nanoparticles genotoxicity evaluation

Nowadays silver nanoparticles (AgNPs) is being widely used in various applications such as in health care items, pipe lines, and washing machines as an antibacterial agent [35, 36]. In wound dressings and catheters silver nanoparticles are used as therapeutic agents. Even then it is controversial for its toxicological effects [37]. Vicia-MCN is carried out to evaluate the genotoxicity of silver nanoparticles. In this assay root tips of *V.faba* is treated with silver nanoparticles in four various concentrations. Finally, the results exposed increased number of chromosomal aberrations (CA), micronuclei (MN) and decreased mitotic index (MI) as a sign of genotoxicity exhibited by the silver nanoparticles. This study confirms that silver nanoparticles is rick for plants and the environment [38]. But seed germination is resistance and root length and mitotic cell cycle were susceptible to Ag NPs [39].

Multi-walled carbon nanotubes (MWCNTs) toxicity evaluation

Multi-walled carbon nanotubes (MWCNTs) along with heavy metals were released from industries. These MWCNTs were investigated using Vicia-MCN, in this study, leaves of *V. faba*was damaged may be due to oxidative stress. It is also predicted that MWCNTs may cause phototoxicity and increases the ecological risks in combination with heavy metals. Vicia-MCNis an implemented as a test plant to determine the toxic effects of MWCNTs released from industries [40].

Cyano bacteria toxicity evaluation

Microcystin-LR (MCY-LR) is a cyanobacteria toxin, in order to find out the toxity caused by it Vicia-MCN was carried out. The findings of the study show MCY-LR is toxic. The mode action could be by altering mitotic activity and inducing mitotic anomalies in the plant *V.faba* [41].

Ionic solvent toxicity evaluation

Genotoxicity and cytotoxicity of ionic solvent, 1-butyl-3-methylimadazolium chloride [(C4MIM)Cl] was studied through Vicia-MCN. This evaluation (C4MIM)Cl exhibits genotoxicity and cytotoxicity of 1-butyl-3-methylimadazolium chloride . This study concluded that Ionic solvent1-butyl-3-methylimadazolium chloride could be unsafe to environment and harmful to human beings [42].

Toxicity Activation

It is believed that metabolites present in *V.faba* activate the herbicides which cause DNA damage. In a study, evaluation of DNA damage of three herbicides, such as, ametryn, metribuzintriazines, and EPTC thiocarbamate was treated with *V.faba* root metabolism in isolated peripheral human lymphocytes using alkaline single cell gel electrophoresis has been carried out. It is been observed in the study that the DNA damage is caused in human peripheral lymphocytes only when herbicides undergo *V.faba* metabolism. There is no damage was observed in control, which does not undergo *V.faba* metabolism. This study reveals that *V.faba* metabolites plays a key role in genotoxicity of herbicides, which induce DNA damage in isolated human peripheral lymphocytes [43].

Breeding analysis

The damage caused by pollutants to plant *V.faba* can be prevented by developing new resistant varieties using breeding methods. Breeding methods can be improved by applying molecular tools. A whole genome analysis is required; it can be done using reliable markers. About 125,559 SSR (Simple Sequence Repeat) sequences were constructed in 32 varieties of faba bean. SSR novel markers are developed in *V.faba* genome using next generation sequencing. These markers can be used for the expression of genetic maps [10]. In a study, *V.faba* and *trancatula*, both the genomes relationships were studied using different markers and no significant differences were observed. This genomic relationship data is a central reference for significance in legumes [44].As 30% to 90% of *V.faba* yields are affected by aschochyte blight in different environmental conditions and found four genomic controlling resistance regions. It is the need of the hour to develop regions which control the resistance [11].*V.faba* is studied for specialized nutrient transfer cell (TC) development, its cells are enriched with transporter proteins that enable nutrient transfer. Among legumes, *V.faba* has largest genome of about 13000 MB. However, only limited amount of genetic information is available. In order to provide a database for future transcript profiling of epidermal TC differentiation this study has been designed to map out de-novo assembly of a genome-wide transcriptome of *V.faba* [45]. The breeding program enhances the quality of faba bean with high nutritional, resistant to stress and pathogens [46].In breeding and out breeding studies on five genotypes of *V.faba* shows depression in first cycle selfing (S_1) and it is not observed in second depression (S_2). RAPD marker analysis showed positive effects, it was not significant to use for evaluating its genetic diversity and in studying faba bean breeding patterns [47]. The mitochondrial DNA (mtDNA) of *V.faba* is about 588,000 bp. Gene sequences of *V.faba* were similar to *Beta vulgaries, Nicotiana tabacum, Vitisvinifera, Oryza sativa, Zea mays and Medicago truncatula.* In which 45% are homologous to *Medicago truncatula*. This breeding analysis may improve the characters of *V.faba* genetically, phenologically, morphologically, physiologically, and biochemical traits make it to adapt to target environments with resistance to biotic and abiotic stresses and understanding its genetic control, establishing a desired variety with weed and nutrient management [48].

Expression of ClpB/Hsp by faba bean

In drought conditions, temperatures were elevated and less rainfall was reported. Plants growing in these harsh places leads to heat stress[49].Heat stress ultimately affects cellular, biochemical and physiologically properties of the plant, which affects the growth of the *V.faba* plant [50, 51].Under such conditions Hsps expresses, in this condition protein misfolds and nonfunctional toxic aggregation takes place [52]. Kumar et al in 2005, initially reported the expression of Hsps in *V.faba*[53]. The expression of ClpB/Hsp 100 was observed in the leaves, seedlings, mature plants, and pollens of the plant. This experiment was carried out under heat stress conditions and the expression studies of ClpB/Hsp 100 were done using semi-quantitative RT-PCR. The RT-PCR was set at 33°C for 2 h and 4 h by using primers complementary to soya bean genome [54].

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