January 2007 *Volume 2 Issue 1*

Trade Science Inc.

Environmental Science

An Indian Journal

Critical Review

ESAIJ, 2(1), 2007 [98-102]

Plant Bioassay: Method For Assessment Of Genotoxicity

Corresponding Author

Parul Singh
Genotoxic Lab,
Dept. of Botany Udai Pratap Autonomous College Varanasi-221002, (INDIA)
E-Mail: parul1780@yahoo.co.in

Co-Authors

Anjil Kumar Srivastava, Ashok Kumar Singh
Genotoxic Lab, Dept. of Botany Udai Pratap Autonomous College Varanasi-221002, (INDIA)

Received: 17th November, 2006
Accepted: 2nd December, 2006

Web Publication Date: 27th December, 2006

ABSTRACT

To prevent the chromosomal abnormalities and DNA damage in plants induced by environmental chemicals and pollutants, it is necessary to understand the outcome of these aberrations and DNA damages that direct to alter the genetic constituents or structures. Higher plants are usual and outstanding tools to detect the genotoxic effect of mutagens and also tremendous indicators of cytogenetic and mutagenic effects of environmental pollution. Due to conserve structure of genetic material, several plants were used as a test material for bioassay. Due to ease and accuracy of detecting and quantifying plant bioassay previously several workers showed that the detection of genotoxicity is simple and does not require costly laboratory outfit. Several advance techniques were used in recent works in place of chromosomal aberration like Micronucleus test, Sister Chromatid Exchange, Comet assay, FISH test, TUNEL test and recently transgenic plants are using as a biomonitoring tool for environmental pollutants and chemicals. The present review article describes the use of different methods of plant bioaasays for the detection of genotoxins.

KEYWORDS

Plant bioassay;
FISH Test;
Comet assay;
TUNEL Test.

INTRODUCTION

Several chemicals pollutants and toxicants are released in environment and exist for long duration in air, water and soil by the activity of industries agriculture and domestic. Several pollutants were reported time to time in air and water which causes the genotoxicity in plants animals as well as human beings. Different industries like dye industry paint industry, metal industry and pesticides industry play

a major role in release of genotoxic chemicals (Genotoxins) in environment. Human beings have been polluting the environment continuously. However in last decades the severity of pollution drastically increased. The increase in concentration of pollutants affects the ecosystem and health of living organisms including human beings[22]. To assess the effect of these pollutants there is a need of precise methods / protocols. Different methods were given time to time by different scientist and workers for assessment of genotoxicity in plants and animals[1,3,13,17].

The plant bioassay used for the assessment of the genotoxicity caused by the pesticides[2] effluents and waste of several industries[22] solid waste[7] waste water[31], mutagens[9] gaseous emission[39] radiations[11,15] and even for assay the potentiality of genotoxicity of drinking water[32]. Genotoxicity is one of the most feared and alarming effects of pollutants for further generation[6]. The utility of plant bioassay to evaluate the genotoxicity of hazardous substances and chemicals is well accepted by different workers[13,17,50]. Allium, Vicia, Hordeum, Solanum and other legumes were used as a plant test material for the bioassay. Low number and large sized chromosome made these plants species ideal for chromosomes studies. The detection of chromosomal aberration is the basic parameter to analyze the genotoxicity but presently some advanced techniques were introduced for the assessment of genotoxicity like Ames test[32], Comet assay[16] TUNEL test, FISH test[48]. Very recently use of transgenic plant introduced to monitor the genotoxicity. In the present study we describe the use of some plant bioassay for the assessment of their genotoxicity caused by the pesticides or pollutants of water, soil and air effluents / sludge of industries and municipal.

Suitable plant material to monitor the genotoxicity

Since[33] introduced the first Allium test, root tip system of various plants like *Allium cepa*[4] and *Allium sativum*[54] *Vicia faba*[26] *barley*[5] and *Tradescantia*[34]. The selection of suitable plant material is very important for the assessment of genotoxicity of chemicals present in air water and soil. The genomic size and chromosome number of plant material is very effective in assessment of genotoxicity. Due to the highly conserved structure of the genetic material it is possible to use a broad variety of species in genotoxicity tests. At present for routine testing bacterial indicators, yeasts, fungi, insects mammalian cells as well as laboratory animals[8]. The plant bioassay plays a very important role for the prediction of health hazards to humans[17,18,40,45,49]. The root meristem cells of plants are sensitive bio indicator of cytogenetic events, as they provide actively proliferating cells and most plant bioassay are well accepted for measuring the genotoxicity of various environmental pollutants.

Chromosomal aberration (CA) and anaphase telophase aberration test

In plants, mainly meristematic root tip cells are used for CA experiments[17,19]. Chromosomal aberrations have been considered as reliable indicators. The analysis of anaphase cells provides additional information on the origin of CAs - breaks; laggards and bridges can be identified at this stage of the cell cycle[49]. The evaluation of CAs is quite time consuming and is facilitated by the use of indicator species that have a small number of large chromosomes. Species, which fulfill these criteria, include *Tradescantia*, *Crepis capillaris*, *Vicia faba*[24] and *Allium cepa*[20]. studied the chromosomal aberrations in Pisum caused by different chemicals. The types and frequencies of chromosomal aberrations were the parameter for the assessment of genotoxicity of a particular chemical. There are several factors affecting the induction of chromosomal aberrations in root meristem cells[25] but the frequency of chromosomal abnormalities in root meristem are mainly depending on the chemical structure and toxicity of chemicals and the duration of treatment.

Micronucleus test

Micronuclei (MN) are formed as a consequence of chromosome breakage (clastogenicity) and disturbance of the spindle apparatus (aneuploidy).The standard procedures of the bioassay were well described in Tradescantia[37,51]. The formation of MN requires cell division and MN are not formed when cell proliferation is delayed. It is important to design test schedules that take account of the cell division time. In the *Tradescantia Micro* nucleus test, a specific

Critical review

stage (early tetrads) is scored and the exposure and recovery time required for the division of the pollen mother cells is included in the experimental design[35,36]. The root tip micronucleus assay replaced earlier chromosomal aberration experiments with plant root cells of *Allium and Vicia*[38]. The scoring of micronucleus in synchronized cell population which is located in the vicinity of the meristmatic section of the root tip is more efficient than the chromosomal aberration assay. The micronucleus assay with the root tips of *Allium cepa* and *Vicia faba* were developed by[12]. The major advantage of this test procedure is that it does not require costly laboratory outfit.

Sister chromatid exchange test

Somatic recombination and sister chromatid exchanges (SCE) can result in chromatid alteration that can affect the expression of genes by the loss of heterozygocity. It has been postulated that specific non-mutagenic carcinogens act via recombinogenic mechanisms. For plants, protocols have been developed for experiments with root cells of *Vicia faba*[9,27] and Crepis capillaries. It is the highly sensitive well known method of assessment to detect the cytological damage. It involves symmetrical exchange at one locus between sister chromatids that does not alter chromosome length and genetic information

The frequency of SCEs per chromosome increases after treatment with genotoxic agents. The plants used for this test should have a low chromosome number and large in size[9].

Single cell gel electrophoresis (SCGE) or Comet assay

The only routine genomic DNA damage test currently carried out with plants is the single cell gel electrophoresis (SCGE or comet) assay. A plant based molecular assay can be applied to detect induce DNA damage[53,43]. It was established for animal cells then it was adapted to plant cells[29]. Along with detection of DNA breaks it also measured the level of DNA migration through an agarose gel in electric field. The comet assay can be used to every plant species[16]. Computerised image analysis system measures the amount of DNA in the head and in the tail and the length of the tail[14]. The DNA damage detected by the comet assay is interesting. The comet assay as first described by[46] and further developed by[47,52] is a sensitive method used for analysis of DNA damage in individual cells. Any nucleated cells can be used for comet assay. It has also relatively low cost.

TUNEL test

TUNEL (TdT mediated dUTP nick end labeling) test is also used to identify the genotoxicity in plants[21] to detect the DNA breaks at a single nucleus, TUNEL test could be used. It takes short time of duration and is easy method for assessment. It is recommended for preliminary evaluation of genotoxicity of genotoxins[23].

AMES Test (Salmone lla assay)

In vitro used very widely as a screening test for evaluation of chemicals[42]. Mostly it is used for monitoring of water system. This test shows high sensitivity and specificity which is the demerit of this system.

FISH test

Fluorescence in situ hybridization is a very useful tool for analysis of chromosome aberration[48]. The traditional and basic method of chromosome staining is not able to detect small changes in morphology or structure of chromosome whereas FISH gives new way to identify the aberrations in genotoxicity[41]. It provides the detection of chromosomal abnormalities in both mitotic as well as interphase nuclei[11,28,44]. It also helps to understand the mechanism of the formation of chromosomal aberrations until now DNA probes for particular plant chromosome are limited; there are few examples where FISH test employed for analysis of chromosome aberrations.

A new approach to monitor the genotoxicity is use of transgenic plants as an indicator[30]. To date, mainly transgenic Arabidopsis and Tobacco plants have been used for the biomonitoring of environmental factors.

REFERENCE

[1] I.D.Adler; Cytogenetic test in mammals, in, S.Vennit, J.M.Parry, (Eds.); 'Mutagenesity Testing-A Practical

Approach', IRL Press, Oxford, D.C.Washington, 275-306 **(1984)**.
[2] S.Ahmad, R.Yasmin; Cytologia, **57**, 155-160 **(1992)**.
[3] B.N.Ames, J.McCann, E.Yamasaki; Mutation Research, **31**, 347-63 **(1975)**.
[4] A.Badr; cytologia, **48**, 451-457 **(1983)**.
[5] B.N.Behera, C.B.Sharma; Biol.Zent.Bl., **109**, 307-311 **(1990)**.
[6] S.Chandra, S.K.Gupta; J.Ecophysiol.Occup.Health, **2**, 225-234 **(2002)**.
[7] S.Chandra, L.K.S.Chauhan, R.C.Murthy, P.N.Saxena, P.N.Pande, S.K.Gupta; Science of the Total Environment, **347**, 46-52 **(2005)**.
[8] L.K.S.Chauhan, P.N.Saxena, S.K.Gupta; Pesti.Biochem.Physiol., **64**, 135 **(1999)**.
[9] F.Cortes, H.C.Andersson; Hereditas, **107**, 7-13 **(1987)**.
[10] F.Cortes, P.Escalza, S.Mateos, M.Diaz Mutation Research, **192**, 125-30 **(1987)**.
[11] I.Dominguez, J.J.W.A.Boei, A.S.Balajee, A.T.Natarajan; Int.J.radiat.Biol., **70**, 199-208 **(1996)**.
[12] H.J.Evans, G.J.Neary, F.Williamson; International Journal of Radiation Biology, **3**, 216-229 **(1959)**.
[13] G.Fiskesjo; Hereditas, **102**, 99-112 **(1985)**.
[14] T.Gichner, J.Veleminsky; Mutation Research, **426**, 163-166 **(1999)**.
[15] T.Gichner, M.Menke, D.A.Stavreva, I.Schubert; Mutagenesis, **15**, 385-389 **(2000)**.
[16] T.Gichner; Mutation research, **538**, 171-179 **(2003)**.
[17] W.F.Grant; Chromosome aberrations assay in Allium A report of USEPA Gene tox. programme. Mutation Research, **99**, 273-291 **(1982)**.
[18] W.F.Grant, M.F.Salamone; Mutation Research, **310**, 187-209 **(1994)**.
[19] W.F.Grant, E.T.Owens; Mutation Research, **410**, 291-307 **(1998)**.
[20] W.F.Grant, E.T.Owens; Mutation Research, **488**, 93-118 **(2001)**.
[21] L.Havel, D.J.Durzan; Bot.Acta, **109**, 268-77 **(1996)**.
[22] W.S.Houk; Mutation Research, **277**, 91-138 **(1992)**.
[23] J.Juchimiuk, J.Maluszynska; Wydawnictwo Uniwersytetu sla skiego., 133-138 **(2003)**.
[24] N.Kanaya, B.S.Gill, I.S.Grover, A.Murin, R.Osiecka, S.S.Sandhu, H.C.Anderson; Mutation Research, **310**, 231-247 **(1994)**.
[25] B.A.Kihlman; J.Biophys. Biochem.Cytol., **2**, 543-545 **(1956)**.
[26] B.A.Kihlman; Mutation Research, **31**, 401-412 **(1975)**.
[27] B.A.Kihlman, H.C.Anderson; Root tips of Vicia faba for the study of the induction of chromosomal aberrations and sister chromatid exchanges, 'Handbook of Mutagenesity Test Procedures', Elsevier, Amsterdam, **1984**, 531-554 **(1984)**.
[28] B.Kolano, E.Wolny, J.Maluszynska; Wydawnictwo Uniwersytetu sla skiego., 139-48 **(2003)**.
[29] G.Koppen, K.J.Angelis; Environ.Mol.Mutagen, **32**, 281-5 **(1998)**.
[30] I.Kovalchuk, O.Kovalchuk, B.Hohn; Trends Plant Sci., **6**, 306-310 **(2001)**.
[31] B.Lah, S.Malovrh, M.Narat, T.Cepeljnik, R.Marinsek-Logar; Environ.Toxicol., **19**, 543-553 **(2004)**.
[32] B.Lah, B.Zinko, M.Narat, R.Marinsek-Logar; Food Technol.Biotechnol., **43(2)**, 139-146 **(2005)**.
[33] A.Levan; Hereditas, **24**, 471-86 **(1938)**.
[34] T.H.Ma, W.F.Grant; Herbarist, **48**, 36-44 **(1982)**.
[35] T.H.Ma, M.M.Harris, V.A.Anderson, I.Ahmed, K.Mohammad, J.L.Bare, G.Lin; Mutation Research, **138**, 157-167 **(1984)**.
[36] T.H.Ma, G.L.Cabrera, A.Cebulska-Wasilewska, F.Loarca, A.L.Vandenberg, M.F.Salamone; Mutation Research, **310**, 211-20 **(1994)**.
[37] T.H.Ma, G.L.Cabrera, B.S.Gill, S.S.Sandhu, A.L. Vandenberg, M.F.Salamone; Mutation Research, **310**, 221-230, **(1994b)**.
[38] T.H.Ma, Z.Xu, C.Xu, H.McConnell, E.V.Rabago, G.A.Arreola, H.Zhang; Mutation Research, **334**, 185-195 **(1995)**.
[39] T.H.Ma, C.Xu, S.Liao, H.McConnell, B.S.Jeong, C.D.Won; Mutation Research, **359**, 39-52 **(1996)**.
[40] T.H.Ma; Mutation Research, **437**, 97-100 **(1999)**.
[41] J.Maluszynska, J.Juchimiuk, E.Wolny; Folia Histochem et Cytobiol, **41**, 101-104 **(2003)**.
[42] D.M.Maron, N.B.Ames; Mutation Research, **113**, 173-215 **(1983)**.
[43] V.J.McKelvey-Martin, M.H.L.Green, P.Schmezer, B.L.Pool-Zobel, M.P.De Meo, A.Collins; Mutation Research, **288**, 47-63 **(1993)**.
[44] A.T.Natarajan, J.J.W.A.Boei, S.Vermeulen, A. S.Balajee; Mutation Research, **372**, 1-7 **(1996)**.
[45] A.T.Natarajan; Mutation Research, **504**, 3-16 **(2002)**.
[46] O.Ostling, K.J.Johanson; Biochem.Bioph.Res. Commun., **123**, 291-298 **(1984)**.
[47] P.L.Olive, J.P.Banath, R.E.Durand; Radiat.Res., **112**, 86-94 **(1990)**.
[48] D.Pinkel, T.Straume, J.W.Gray; Proc.Natl.Acad. Sci.USA, **83**, 2394-2398 **(1986)**.
[49] J.Rank; The Method of Allium Anaphase-Telophase Chromosome Aberration Assay, **(2003)**.
[50] J.Rank, M.H.Nielsen; Mutation Research, **418**, 113-119 **(1998)**.
[51] G.S.Rodrigues, T.H.Ma, D.Pimental, L.H.Weinstein;

Critical review

Crit.Rev.Plant Sci., **16**, 325-359 **(1997)**.

[52] N.P.Singh, M.T.McCoy, R.R.Tice, E.L.Schneider; Exp.Cell Res., **175**, 184-191 **(1988)**.

[53] R.R.Tice, P.W.Andrews, O.Hirai, N.P.Singh; Adv.Exp. Med.Biol, **283**, 157-164 **(1991)**.

[54] P.Vera Lopez, C.Ruiz Rejon, R.Lozano, M.Ruiz Rejon; Cytobios, **62**, 135-139 **(1990)**.