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# MOLECULAR BASED RAPID METHOD TO RESOLVE THE EXTENT OF DNA DAMAGES IN *ACALYPHA INDICA*

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## ABSTRACT

The comet assay is a comparatively simple, but sensitive and well validated tool for measuring strand breaks in DNA in single cells. Cells were embedded in a thin layer of agarose on a microscope slide and lysed with detergent and high salt solution. Air pollution tolerance index of *Acalypha indica* growing in the natural conditions closest to road side and non road side were determined by comet assay.

**KEYWORDS:** **DNA Damage, Plants, Bioindicators, *Acalypha indica***

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# INTRODUCTION

Air pollutants generated from traffic and industrial plants are believed to be one of the major causes of DNA damage in living species. Effects of the combination of these pollutants on living organisms have not been clarified in detail. In many cases, some indicator plants have been chosen and their responses to stress agents have been evaluated. Particularly, their biomass values followed by the harvest were considered at first place. For example, bioindicators exposed to air pollutants and natural environmental stresses such as water stress, nutrient deficiency and temperature stress may undergo some biochemical and physical alterations. However, some of which are able to accommodate large amount of pollutants without undergoing damage. In this case, only a chemical analysis may allow the determination of toxic elements. However, in the growth season no valid tests, especially non-destructive tests, have been carried out *in situ* conditions. In some cases, biological stress agents such as fungi or bacteria might also suffer from the environmental stresses while they are infecting the host (Dikilitas, 2003).

Plants are exposed to various types of environmental xenobiotics. Assays to detect the genotoxicity of these xenobiotics are at present not available for most plant species. This limitation hampers or prevents the detection of the genotoxicity of environmental xenobiotics in plants growing for example, on polluted soil. To overcome this limitation, a plant based molecular assay – the COMET assay, can be applied to detect induced DNA damage (Tice et al 2002, Collins 2004). Although this technique has been primarily applied to animal cells, the incorporation of the comet assay with plant tissues (Koppen and Verschaeve 1996, Gichner and Plewa 1998) significantly extends the utility of plants in basis and applied studies in environmental mutagenesis. In theory, the comet assay can be used to every plant species.

The comet assay or single-cell gel (SCG) test is a micro gel electrophoresis technique that measures DNA damage at the level of single cells. A small number of cells suspended in a thin agarose gel on a microscopic slide is lysed, electrophoresed, and stained with a fluorescent DNA binding dye. Cells with increased DNA damage display increased migration of chromosomal DNA from the nucleus toward the anode, which resembles the shape of a comet. In its alkaline version, which is mainly used, DNA single-strand breaks, DNA double-strand breaks, alkali-labile sites, and single-strand breaks associated with incomplete excision repair sites cause increased DNA migration. On the other hand, cross links (DNA-DNA or DNA-protein) can lead to decreased DNA migration. Variations of the comet assay have been established for the detection of specific DNA base modifications (Speit G, Hartmann A, 2005).

The efficiency of plants in absorbing pollutants in such that it can produce pockets of clean air (Gilbert 1968). Bernatsky (1969) has suggested that green belts might help to reduce air pollution. Plants growing in air polluted environment often responded and showed significant changes in their morphology, physiology and biochemistry.

Plant bioassays are cost and time effective and do not require specific equipment, excessive sample manipulation, and concentration procedures and have been used successfully for in situ exposure studies. For example, *Vicia faba* and *Arabidopsis thaliana* were used to monitor the genotoxic effects (sister chromatid exchanges and micronuclei) in air and soils polluted by industrial factories (Chroust et al., 1997), and single cell gel electrophoresis assays (SCGE) with ginkgo, pohtos and periwinkle were successfully employed in experiments to study air pollution caused by traffic emissions (Sriussadaporn et al., 2003). Furthermore, methods have been developed with bioindicators for the detection of specific air pollutants such as fluorides

(Weinstein and Davison, 2003) and ozone (Bytnerowicz et al., 2002; Manning et al., 2002; Manning, 2003).

This study aims to detecting the genetic damages in *Acalypha indica* present in residential and roadside environments. The samples were analyzed by using cometT assay. This study might be applied as a preliminary method in urban air quality assessment for detecting the existing effects of air pollutants and micro-environmental stress in an urban ecosystem.

## MATERIALS AND METHODS

### *Collection of Plant Material*

*Acalypha indica* plant sample was collected from Namakkal District, Tamilnadu, India. Samples were immediately transported to the laboratory.

### *Isolation of nuclei from plant callus or cell cultures*

The cell suspensions of leaves were prepared and poured onto a pad of multiple layers of cheese-cloth. The cells were rinsed with a phosphate buffer pH.7 and scraped the cells from the cheese-cloth and transferred to a micro centrifuge tube. The cells were gently agitated with small amounts of sterile sands and buffer. After the settlement, the mixture was filtered through the mesh nylon filter to obtain the nuclei and then kept it in a ice bath for the comet assay (Stavreva and Gichner, 2002).

### *Slide Preparation*

Microscopic slides with frosted ends were placed on a neat platform. The cells were suspended in 1% of N-Methylpthalic acid (NMPA). The 1% of agarose was prepared and kept it in warm condition, then the cells were added with the molten agrose. Then immediately 4 μl of Etbr was added to the agarose and mixed well. This mixture was poured evenly on the clean frozen slides and kept it frozen temperature(-20°C) for 10 minutes for alkali unwinding of DNA.

### *Electrophoresis*

After alkali unwinding, the single-stranded DNA was electrophoresed under alkaline conditions for 45 minutes to produce comets. The gels were neutralized by rinsing the slides at least three times with the Tris (pH 7.5) buffer such as for 5 min each during electrophorosis(Rojas et al., 1999; Kocyigit et al., 2005; Gichner et al., 2008). After electrophoresis, the slides were neutralized with a neutralization buffer (0.4 mol $L^{-1}$ Tris-HCl, pH 7.5) at room temperature for 15 min.

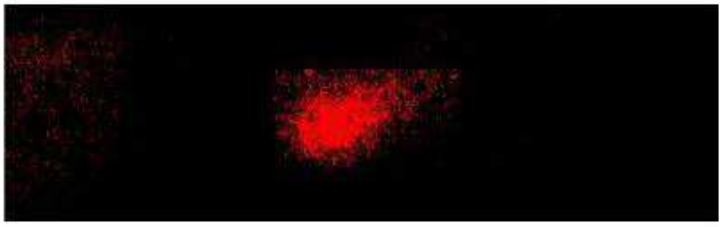
## RESULTS AND DISCUSSION

*Acalypha indica* collected from two different places were analyzed. One from the roadside which was highly polluted with the vehicle exhaust and the other from the residential area with less vehicle exhaust pollution. DNA damage in *Acalypha indica* was observed by Single cell gel electrophoresis (SCGE)(Figure I). The nuclei collected from the plants located in residential area exhibited no DNA migration and failed to provide information about the extent of migration among more damaged cells. Nuclei from the roadside plants exhibited various length of DNA migration indicating the presence of damaged DNA due to vehicle exhaust pollution.

**Figure I (A&B)**
***Photomicrographs of EtBr-stained DNA from protoplasts of Acalypha indica processed for alkaline comet assay. DNA Damage Tail moments***

**(A)**

**(**B**)**



The comet assay or single-cell gel electrophoresis (SCGE) has become one of the standard methods for assessing DNA damage. The assay attracts adherents by its simplicity, sensitivity, versatility, speed and economy. Although it is essentially a method for measuring DNA breaks, the introduction of lesion – specific endonucleases allows detection of, for example, ultraviolet (UV)-induced pyrimidine dimers, oxidized bases and alkylation damage. The comet assay is most commonly applied to animal cells, whether in culture or isolated from the organism (eg., Lymphocytes separated from blood or cells from disaggregated tissues). However, methods have also been developed to examine damage in the DNA of plant cells. The cellulose plant cell wall presents a barrier to the release of DNA to form a comet tail, but physically chopping up the tissue with a razor blade releases nuclei that can then be embedded in agarose (Collins 2004).The plant cell has a wall that cannot be removed has the animal cell membrane by lysis in high concentrations of detergents and salts and so the nuclei have to be isolated mechanically. It is exceedingly important to gently isolate the nuclei from the leaves (Gichner T 2008). The clean frosted slides were used for coating the nuclei suspended in agarose. Fully frosted slides were used most commonly because they offered increased gel bonding and thus stability.

## CONCLUSION

There are numerous software packages to choose from, that will compute fluorescence parameters for comets selected by the operator. The most commonly used parameters are tail length, relative fluorescence intensity of head and tail and tail moment. It is also possible to compute DNA damage from comets without sophisticated image analysis programs. The human eye is easily trained to discriminate

degrees of damage according to comet appearance (Collins 2004). The Nuclei collected from the plants located in residential area exhibited no DNA migration and failed to provide information about the extent of migration among more damaged cells. Whereas nuclei from the roadside plants exhibited various length of DNA migration *indica*ting the presence of damaged DNA due to vehicle exhausts pollution.

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