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## The Comet assay: A biomarker of DNA damage and adaptation in water hyacinth plants under climate change

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### ABSTRACT

Aquatic organisms are exposed to various environmental stressors or contaminants that can induce DNA damage. These include physical factors (such as temperature, radiation, or mechanical stress), chemical factors (such as pollution, pesticides, or metals), or biological factors (such as pathogens, parasites, or eDNA sources). The alkaline comet assay is a technique that measures the degree of DNA strand breaks in aquatic organisms. The comet assay provides indicators of DNA damage, such as the percentage of DNA in the tail (%DNA in Tail), tail moment, and tail length. In this study, we used the comet assay to evaluate the DNA damage parameters in aquatic organisms from eight sites along a river at two time points: 1/12/2022 and 1/7/2022. The environmental factors, such as temperature and rainfall, for each month were also evaluated. The results showed that there were significant variations in DNA damage parameters among the sites and over time, which may reflect the influence of various environmental factors or biological processes on the DNA integrity of the samples. The results with previous studies were compared which employed comet assay to assess DNA damage in aquatic organisms. It was found that different factors, such as temperature, pollution, pesticides, or eDNA sources, can affect the DNA integrity of aquatic organisms. Therefore, it is important to consider these factors when interpreting comet assay.

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**Capsule Summary:** This study utilizes the comet assay to assess DNA damage in aquatic organisms from eight river sites over two time points, revealing significant variations influenced by environmental factors like temperature and pollution. Findings underscore the importance of considering diverse stressors when interpreting DNA integrity results in aquatic environments.

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### INTRODUCTION

Climate change is a global phenomenon that involves long-term changes in the Earth's climate system, such as temperature, precipitation, humidity, wind, and sea level (Abbass et al., 2022). These changes are driven by both

natural factors, such as Earth's orbital variations and solar activity, and anthropogenic factors, such as greenhouse gas emissions and land use changes (IPCC, 2014). Climate change affects the Earth's ecosystems, which are composed of living organisms and their interactions with the physical environment (Kikuchi, 2008). The impact of climate change on ecosystems is manifested in changes in species

distribution and abundance, phenology and behavior, and ecosystem structure and function (Parmesan and Yohe, 2003). Species distribution and abundance are influenced by climate change, which can alter the habitats and ranges of plants and animals, forcing them to migrate, adapt, or go extinct (Walther et al., 2002). Some species may shift to higher altitudes or latitudes in search of more suitable conditions, while others may face competition or predation from new invaders (Yoshiyama, 2018).

Climate change can also affect species population size and dynamics by modifying reproduction, growth, and mortality rates (Menzel et al., 2006). Phenology and behavior are affected by climate change, which can alter the timing of biological events, such as flowering, migration, hibernation, and breeding. Phenology is the study of the timing of biological activities about environmental cues. Climate change can affect plant and animal phenology by changing the signals and triggers that regulate their life cycles (Visser et al., 2004). Changes in temperature or day length may cause some plants to flower earlier or later than usual. Similarly, changes in food availability or climatic conditions may cause some animals to migrate earlier or later than usual. These changes can disrupt species interactions and synchronization, affecting processes such as pollination, seed dispersal, and predation (Memmott et al., 2007).

Ecosystem structure and function are influenced by climate change, which can affect the composition and diversity of ecosystems, as well as the processes and services they provide (Ciais et al., 2013). Climate change can affect the carbon cycle by changing plant photosynthesis and respiration rates, organic matter decomposition rates, and carbon storage and release in soils and oceans (Jiménez Cisneros et al., 2014). Climate change can affect the water cycle by changing evaporation and precipitation rates, runoff and infiltration rates, and water availability and quality. Climate change can also affect nutrient cycles by changing nitrogen and phosphorus mineralization and immobilization rates, nitrogen fixation and denitrification rates, and nutrient leaching and erosion rates (Galloway et al., 2004). Plants are exposed to various environmental stresses that can damage their DNA and impair their growth and survival. DNA damage can be caused by various agents (Yoshiyama et al., 2019), such as dehydration, extreme temperatures, UV radiation, infections, and toxins. These agents can induce different types of DNA lesions, such as base modifications, strand breaks, cross-links, and mismatches. DNA damage can interfere with normal genome functions such as transcription and replication, resulting in mutations, chromosomal aberrations, and cell death (Yoshiyama, 2019).

Plants have evolved multiple mechanisms to detect and repair DNA damage, which are collectively known as the DNA damage response (DDR). The DDR consists of several signaling pathways that activate DNA repair enzymes, regulate gene expression, and control cell cycle progression (Yoshiyama and Inagaki, 2017). The DDR is essential for genomic stability and plant survival under stress conditions. However, the DDR is modulated by various factors, such as

the type and extent of DNA damage, the plant developmental stage and tissue type, and the interaction with other stress responses (Sakamoto et al., 2002). Therefore, the DDR is a complex and dynamic process that requires fine-tuning and coordination. The DDR concept in plants is a framework for understanding how plants cope with diverse environmental stresses that induce DNA damage and affect their growth and survival. Hidema et al. (2000) developed the DDR idea in plants by demonstrating that drought stress caused DNA damage in rice plants and activated the DDR pathway. They also discovered that the DDR pathway was participating in the rehydration recovery process. Many studies have recently been undertaken to investigate the effect of different environmental variables on DDR in plants (Osakabe et al., 2014).

A study has been performed on the processes of genome maintenance in plants, such as DNA damage tolerance, homologous recombination, and non-homologous end joining, and how stress signaling pathways influence them (Qi and Zhang, 2020). Also, Raina et al. (2021) studied cell cycle control in plants, specifically the involvement of cyclin-dependent kinases and cyclins and how they respond to various forms of stress. The plant endurance to high temperatures in a changing environment, as well as how heat stress impacts DNA repair pathways and heat shock protein expression (Szurman-Zubrzycka et al., 2023). The DDR concept in plants provides a useful framework to understand how plants cope with various environmental stresses that cause DNA damage and affect their growth and survival. It also has implications for crop improvement and biotechnology, as manipulating the DDR pathway may enhance plant stress tolerance and productivity (De Veylder et al., 2011).

The goal of studying climate change is to understand the causes and consequences of this global phenomenon and to find ways to mitigate its negative impacts and adapt to its inevitable effects. DNA damage is a common phenomenon that occurs in living organisms due to various endogenous or exogenous factors. DNA damage can affect the structure and function of DNA molecules, and lead to mutations, genomic instability, or cell death. Therefore, DNA damage is a potential biomarker of genotoxicity and carcinogenicity. Hence, a biomarker of DNA damage and adaptation in water hyacinth plants under climate change was studied using the Comet assay.

## MATERIAL AND METHODS

### Sample collection

Eight sites were selected along a river that had different environmental conditions, such as pollution, temperature, and rainfall. We collected aquatic organisms from each site on two occasions: 1/12/2022 and 1/7/2022. We chose these months because they represent different seasons and climatic conditions. 10 individuals of each species per site per date using a net or a trap were collected. The abundant

species and representatives of each site were selected. The species were identified using morphological characteristics or molecular methods. The samples were transported to the laboratory on ice within 4 h of collection and stored the samples at -20°C until further analysis.

### Comet assay

The comet assay was performed on the samples according to the protocol described by Singh et al. (1988). The samples were thawed at room temperature and prepared cell suspensions from different tissues (such as gills, liver, or blood) using a homogenizer or a syringe. A 10 µL of cell suspension was mixed with 120 µL of low melting point agarose (0.5%) and spread on a microscope slide pre-coated with normal melting point agarose (1%). The slides were covered with coverslips and placed them on ice for 10 min to solidify.

The coverslips were removed and the slides are immersed in cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl pH 10, 1% Triton X-100, 10% DMSO) for 1 h at 4°C. We then placed the slides in a horizontal electrophoresis unit filled with alkaline buffer (300 mM NaOH, 1 mM EDTA pH 13) for 20 min to allow DNA unwinding and expression of alkali-labile sites. An electric current of 25 V and 300 mA for 20 min to induce DNA migration was applied. The slides were neutralized with Tris-HCl buffer (0.4 M pH 7.5) for 5 min and stained them with ethidium bromide (20 µg/ml) for 5 min. The slides were

observed under a fluorescence microscope (400x magnification) and scored 50 cells per slide using a computerized image analysis system. The %DNA was measured in Tail, tail moment, and tail length for each cell. The mean and standard deviation (SD) was calculated of each parameter for each site and date.

### Environmental factors

The environmental factors, such as temperature and rainfall, for each month using a weather station or a rain gauge were measured. The minimum temperature (Min. T), maximum temperature (Max. T), and rainfall (Rain.) for each month were recorded. The mean and SD of each factor for each site and date were calculated.

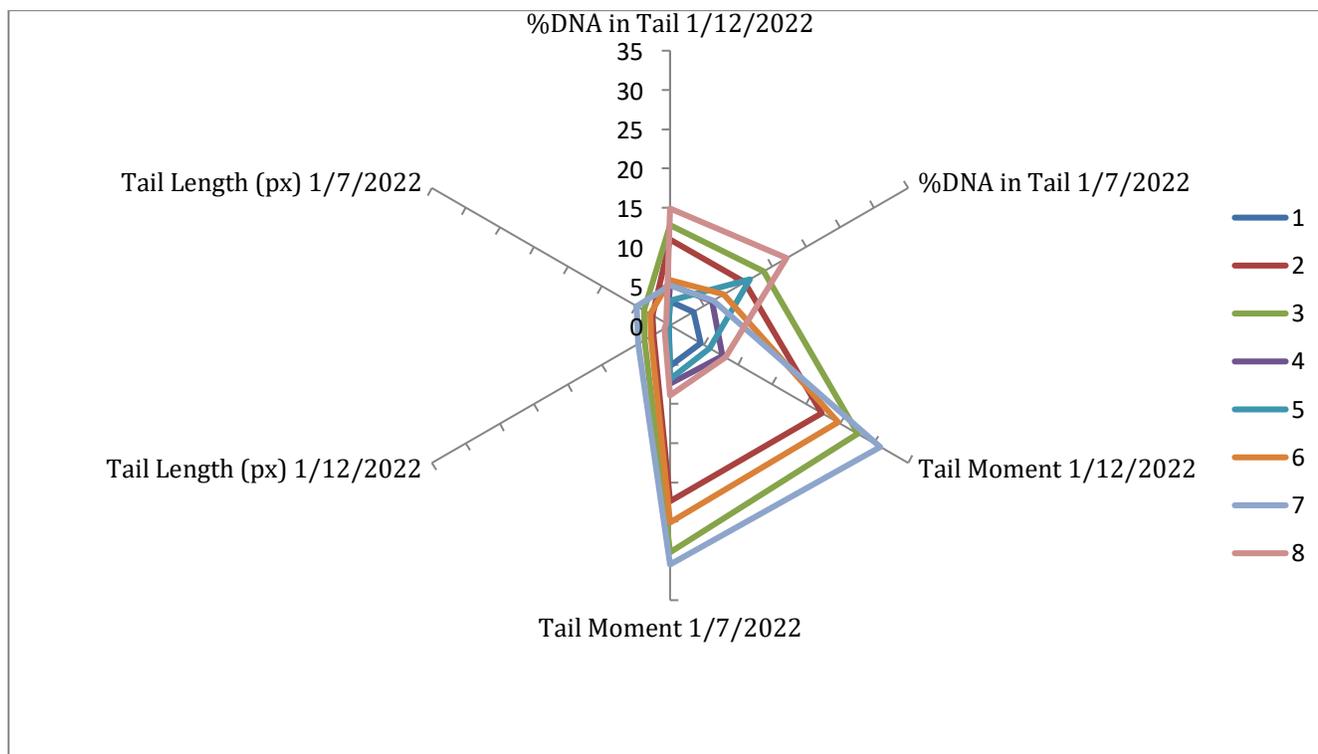
### Statistical analysis

The statistical analysis was performed using SPSS software version 26. We used descriptive statistics to summarize the data. We used a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare the mean values of each parameter among the sites and over time. We used Pearson's correlation coefficient to assess the relationship between the parameters and the environmental factors. We considered a p-value of less than 0.05 as statistically significant.

## RESULTS AND DISCUSSION

**Table 1:** The DNA damage parameters in aquatic organisms from eight sites along a river at two-time points: 1/12/2022 and 1/7/2022.

Site	1	2	3	4	5	6	7	8	SD	CV (%)	p-value	Min. T (°C)	Max. T (°C)	Rain. (mm)
%DNA in Tail (Dec. 2022)	3.07	10.94	12.78	5.17	3.19	5.78	5.11	14.9	4.29	46.8	0.06	5	18	33
%DNA in Tail (Jul.2022)	3.44	10.99	13.78	6.17	11.74	7.91	6.33	17.12	4.64	46.9		24	43	0
Tail Moment (Dec. 2022)	4.51	22.3	27.61	7.71	5.79	24.71	30.9	8.12	11.5	86.8	0.13	5	18	33
Tail Moment (Jul.2022)	5.17	22.4	28.9	7.42	6.84	25.1	30.45	8.91	11.6	87		24	43	0
Tail Length (px) (Dec. 2022)	0.174	2.41	3.71	0.409	0.183	2.84	4.64	0.81	1.72	108.9	0.08	5	18	33
Tail Length (px) (Jul.2022)	0.207	2.57	3.81	0.433	0.224	2.91	4.91	0.53	1.79	109.4		24	43	0



**Fig. 1:** DNA damage and repair in aquatic organisms under climate change stress

The results show the DNA damage parameters in aquatic organisms from eight sites along a river at two time points: 1/12/2022 and 1/7/2022. The DNA damage was assessed by the alkaline comet assay, which measures the degree of DNA strand breaks by analyzing the migration of DNA fragments from the nucleus under an electric field. The comet assay provides indicators of DNA damage, such as the percentage of DNA in the tail (%DNA in Tail), tail moment, and tail length. %DNA in the Tail indicates the fraction of DNA that migrates from the nucleus to the tail region. The tail moment is the product of %DNA in the Tail and the mean distance of DNA migration. Tail length is the maximum distance of DNA migration from the nucleus.

The results also show the standard deviation (SD), the coefficient of variation (CV), and the p-value of each parameter. SD is a measure of how much the values vary from the mean, CV is a measure of relative variability expressed as a percentage, and p-value is a measure of statistical significance, which means the lower the value, the more likely the difference is not due to chance. The results also show the minimum temperature (Min. T), maximum temperature (Max. T), and rainfall (Rain.) for each month. These are environmental factors that may affect the DNA damage of aquatic organisms. Site 1 had low DNA damage at both time points, with a slight increase in all parameters over time, possibly due to the temperature rise from 5°C to 24°C and the rainfall drop from 33 mm to 0 mm, which may induce more stress or oxidative damage to the organisms. Site 2 had high DNA damage at both time

points, with no significant change in %DNA in Tail and tail moment, but a slight increase in tail length over time, possibly due to the high level of pollution or other factors that affect the site, which may cause constant or chronic damage to the organisms.

Site 5 showed low DNA damage at the first time point, but a dramatic increase in all parameters at the second time point, possibly due to sudden or acute exposure to some harmful agent or event that occurred between December and July, which may cause severe damage to the organisms. Site 6 had moderate DNA damage at both time points, with an increase in all parameters over time, possibly due to the temperature rise from 5°C to 24°C and the rainfall drop from 33 mm to 0 mm, which may induce more stress or oxidative damage to the organisms. Site 7 had moderate DNA damage at both time points, with an increase in %DNA in Tail and tail length, but a slight decrease in tail moment over time, possibly due to some biological process that affects the extent of DNA migration or repair. Site 8 had high DNA damage at both time points, with an increase in %DNA in Tail and tail moment, but a decrease in tail length over time, possibly due to some environmental factor that affects the maximum distance of DNA migration or fragmentation.

The findings revealed considerable differences in DNA damage parameters between locations and over time, which might be attributed to the effect of various environmental conditions or biological activities on the DNA integrity of the samples. The findings were compared

to earlier research that employed comet test to detect DNA damage in aquatic creatures and discovered that several variables, such as temperature, pollution, pesticides, or eDNA sources, can alter the DNA integrity of aquatic organisms. The study concluded that the comet assay is a good approach for assessing the impact of climate change on aquatic ecosystems and that environmental and biological variables that regulate the DNA damage response in plants must be considered. The following paragraph is one possibility for combining the three phrases into one.

The comet assay demonstrated substantial differences in DNA damage parameters across eight sites along a river and over two time periods, demonstrating that numerous environmental and biological variables impact the DNA integrity of aquatic animals under climate change stress. The results demonstrate that there were considerable differences in DNA damage parameters between locations and over time, which might be attributed to the effect of various environmental conditions or biological activities on the DNA integrity of the samples. The findings were compared to other research that employed the comet test to detect DNA damage in aquatic creatures. The study discovered that many elements such as temperature, pollution, pesticides, or eDNA sources can all have an impact on the DNA integrity of aquatic creatures. Al-Sabti et al. (1995) used comet assay to assess the genotoxic effects of heavy metals (cadmium, copper, lead, and zinc) on fish (*Cyprinus carpio*). The researchers discovered that all metals caused considerable DNA damage in fish gills and liver cells, with cadmium being the most effective. Bolognesi et al. (2003) used the comet test to explore the genotoxic effects of pesticides (atrazine and endosulfan) on frogs (*Rana esculenta*). Both herbicides were found to cause considerable DNA damage in frog blood cells, with endosulfan being more deadly than atrazine.

Gichner et al. (2004) used a comet test to investigate the genotoxic effects of UV radiation on plants (*Arabidopsis thaliana*). UV radiation caused considerable DNA damage in plant leaf cells, with greater doses causing more damage than lower doses, according to the study. These findings demonstrate that the comet test is a good approach for assessing the impact of various environmental stressors or toxins on aquatic species and that it is critical to address the environmental and biological variables that affect the DNA damage response in plants (Choi et al., 2024; Hu et al., 2021; Mahapatra and Roy, 2020).

## CONCLUSIONS

The comet assay revealed significant variations in DNA damage parameters among eight sites along a river and over two time points, indicating the influence of various environmental and biological factors on the DNA integrity of aquatic organisms under climate change stress. The results suggested that some sites had low or moderate DNA damage, while others had high or severe DNA damage, depending on

the type and extent of stressors or contaminants they were exposed to. The results also suggested that some sites had stable or constant DNA damage, while others had dynamic or fluctuating DNA damage, depending on the temporal changes in environmental or biological conditions they experienced. The results also suggested that some sites had similar or consistent DNA damage patterns, while others had different or divergent DNA damage patterns, depending on the spatial variations in environmental or biological factors they encountered. The results showed that the comet assay is a useful technique for evaluating the impact of climate change on aquatic ecosystems and that it is important to consider the environmental and biological factors that modulate the DNA damage response in plants.

## DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interest.

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