Telomere Length Profiling in Arabidopsis Thaliana Under Environmental Stress Using Real-Time PCR

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ABSTRACT

The research examined the influence of environmental stress induced by the presence of heavy metals, to which the organism is exposed at varying concentrations, and its effect on telomere length in Arabidopsis thaliana. The study tested five heavy metals which included copper (II) chloride, cadmium (II) chloride, ferric chloride, magnesium sulfate and lead (II) nitrate. The plants received their nutrients from Murashige and Skoog (MS) culture medium that contained each metal solution. The research used four different concentration levels of 1 ppm, 5 ppm, 10 ppm and 20 ppm for each metal. The cultivation was carried out under sterile conditions, and the plants were exposed to a photoperiod of no less than 16 hours per day at a temperature ranging between 23–24 °C. Special attention was given to maintaining low humidity levels, as this species is highly sensitive to moisture. Experimental observations confirmed that elevated humidity can significantly hinder plant growth. The growth period lasted for 48 days until researchers collected plant tissue samples. Real-time PCR analysis showed that most heavy metal concentrations above 5 ppm resulted in longer telomeres when compared to lower concentration plants. The plant activates an early defense response when facing stressful environmental conditions which leads to this observed phenomenon. The telomere length decreased in plants treated with elevated copper and magnesium concentrations indicating negative effects from these metal levels. The culture media with high iron concentrations (10 ppm and 20 ppm) showed no germination at all, which may indicate that iron exerts a strongly negative effect on the growth of this plant.

KEY WORDS

Arabidopsis thaliana, Environmental Stress, heavy metals, qPCR, Telomer length.

INTRODUCTION

Environmental pollution stands as a significant worldwide issue because it severely affects both ecosystems and human wellness, The increase in human activities during the past decades has led to environmental pollution through the release of various pollutants and heavy metals represent some of the most dangerous among them [1] Heavy metals which have a density above 5 g/cm³ create severe threats to environmental systems and all living organisms, Cadmium and Lead are among the most dangerous heavy metals because they dissolve easily in water systems which enables their absorption and bioaccumulation leading to detrimental biological effects[2] . The exposure to heavy metals such as lead and cadmium leads to significant telomere shortening because of their chronic nature, The elevated oxidative burden generated by these metals leads to gradual cellular integrity breakdown which causes this phenomenon [3]. The protective caps known as telomeres prevent cells from mistakenly activating repair mechanisms which could result in harmful chromosomal fusions that endanger genomic stability essential for life [4][5]. The harmful effects of lead and cadmium on telomere integrity have

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been extensively documented in humans and other organisms, these metals cause redox imbalance and DNA damage, both of which are central contributors to telomere attrition. Given the evolutionary conservation of stress response pathways, it is plausible that similar mechanisms operate in plants like Arabidopsis thaliana, where telomere length may also be negatively influenced, ultimately jeopardizing genomic stability [6][7]. Heavy metals cause disruptions in Arabidopsis thaliana growth and development which may indirectly affect telomere dynamics, these disruptions not only impact physiological functions but may also reflect deeper cellular consequences, including those related to telomere maintenance, highlighting the plant's complex and sensitive response to toxic environmental challenges [8]. The accumulation of excess iron leads to increased oxidative stress which drives telomere shortening through the generation of reactive oxygen species (ROS) that specifically target guanine-rich sequences of telomeric DNA, this oxidative damage compromises telomere integrity and accelerates cellular aging, underscoring the delicate balance required for maintaining genomic stability in the face of metal-induced stress [9]. The structural integrity and functional stability of telomeres depends on magnesium because this essential cofactor enables various enzymatic processes that control DNA replication and repair thus maintaining telomeres properly the presence of magnesium is not merely supportive; it is foundational [10]. Cadmium primarily affects plant growth by promoting reproductive development but simultaneously causes damage to vegetative growth and affects Arabidopsis thaliana dynamics through substantial impacts on its physiological and developmental processes [11]. The exposure of Arabidopsis thaliana to lead results in ROS production that subsequently generates oxidative damage to DNA and other cellular components [12]. The exposure to lead is a well-recognized factor that contributes to telomere erosion, and this has also been observed in human studies where lead exposure is associated with reduced telomere length and elevated markers of oxidative disruption [13][14]. The aim of the current study to assess the influence of some heavy metals (copper (II) chloride, cadmium (II) chloride, ferric chloride, magnesium sulfate, and lead (II) nitrate) in telomer length of Arabidopsis thaliana as a genetic mutation.

MATERIALS AND METHODS

The practical experiment was conducted in the Tissue Culture Laboratory, Department of Biology, College of Science, University of Mosul.

Plant: The experiment used Arabidopsis thaliana CS70000 which were obtained from the Arabidopsis Biological Resource Center (ABRC) at The Ohio State University, USA.

Cultivation: Seeds grown in Murashige and Skoog (MS) medium. The seedlings received continuous light at 23–24°C temperature conditions for at least 16 hours daily to create an optimal growth environment. The evaluation included five heavy metal salts which were copper (II) chloride, cadmium (II) chloride, ferric chloride, magnesium sulfate and lead (II) nitrate. The standard dilution formula was used to create MS media solutions at 1 ppm, 5 ppm, 10 ppm and 20 ppm concentrations from their concentrated salt solutions. The experiment required sterile cultivation procedures to preserve both experimental accuracy and biological material integrity. The cultivation process lasted 48 days from seed planting until the plants reached maturity before sample collection for analysis.

Polymerase chain reaction (PCR): The Polymerase Chain Reaction (PCR) represents a revolutionary laboratory method which has transformed scientific research through its

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ability to duplicate DNA sequences outside living organisms, The technique serves as a robust method for studying genetic pathogen origins and population genetic polymorphisms which enhances our knowledge of molecular biology and disease mechanisms [15]. The experiment used real-time digital polymerase chain reaction (digital PCR) to determine telomere length because this method provides both high throughput and precise absolute telomere length measurement, The technique provides valuable detection of telomere length distribution patterns which enhances our understanding of cellular aging and genomic stability through its reliable and sensitive approach [16 [:

The samples were prepared and the required tests were conducted in accordance with the instructions provided in the Genomic DNA Mini Kit (Plant) protocol.

Primer design: The primer of the telomere with the specific primer of the housekeeping gene according to this study [17] shown in the following

Table 1: primers that used in the study

Sequence	Primer
5'- CCCCGGTTTTGGGTTTTGGGTTTTGGGTTTTGGGT-3'	Tel: A Forward primer
5'- GGGGCCCTAATCCCTAATCCTAATCCCCCTAATCCCT-3'	Tel: B Reverse primer
5'-CGGCGGCGGGCGGCGCGGGCTGGGGGGGGGAGAAGACACAAATGGTTCGC-3'	Forward housekeeping gene
5'-GCCCGGCCCGCCGCGCCCGTCCCGCCGTCCATTCCTTGCACCACTTTC-3'	Reverse housekeeping gene

Calculate the rate of gene expression :

The gene expression rate of the genes was calculated based on the CT value of the target gene with the housekeeping gene for the control and plant samples using the following equation [18:[

:1ΔCT (Experimental sample) = CT (Target gene of the experimental sample) - CT (Reference gene for the experimental sample .(

ΔCT (control) = CT (Target gene of the control) - CT (Reference gene for the control.(

CT (Target gene of the experimental sample) refers to the number of DNA cycles for the telomere genes in experimental plant samples.

CT (Reference gene for the experimental sample) indicates the number of DNA cycles for the housekeeping gene in experimental plant samples.

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CT (Target gene of the control) refers to the DNA cycles of the Telomer genes for control plant samples.

CT (Reference gene for the control) refers to the DNA cycles of the housekeeping gene for control plant samples.

:2The equation of ΔCT for the treated sample relative to ΔCT for the control sample was calculated according to the following :

ΔΔCT =ΔCT (Experimental sample) -ΔCT) control(

:3The gene expression value is calculated according to the following equation: Gene Expression Folding.

Gene Expression Folding = 2 -ΔΔCT

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RESULTS AND DISCUSSION

Real-time PCR analysis followed by telomere length calculation through established equations produced results which differed from what was anticipated at first. The telomere length measurements of lead-treated plants showed positive results at 1 ppm but telomere lengths decreased with increasing lead concentrations. The highest concentration of 20 ppm led to telomere lengths that surpassed all previous measurements. The telomere length measurements in copper-treated plants followed a predictable sequence. The lowest copper concentration led to the longest telomeres which shortened as copper levels increased. The telomere shortening observed in plants exposed to high copper levels matches expected biological reactions to heavy metal stress. The exposure of plants to cadmium and iron resulted in telomere length patterns that mirrored lead exposure effects while magnesium-treated plants displayed copper-like telomere length changes. The heavy metal-exposed plants demonstrated longer telomeres than the standard control plants in all cases. The unexpected pattern of longer telomeres in metal-exposed plants indicates a sophisticated biological response to metal stress which reflects plants' ability to adapt under difficult environmental conditions.

*The value in fold change represents the telomere length.

Table 2: This table indicates the treatments exposed to a concentration of 1 ppm.

Fold Change (2^-ΔΔCT)	**Added metal**
55.43	**Pb**
3.2042	**Cd**
27.99	**Mg**
35.22	**Cu**
26.58	**Fe**

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Table 3: This table indicates the treatments exposed to a concentration of 5 ppm.

Fold Change (2^-ΔΔCT)	Added metal
10.88	**Pb**
2.4283	**Cd**
16.52	**Mg**
18.32	**Cu**
210.56	**Fe**

Table 4: This table indicates the treatments exposed to a concentration of 10 ppm.

Fold Change (2^-ΔΔCT)	Added metal
42.42	**Pb**
24.9332	**Cd**
15.91	**Mg**
6.4085	**Cu**
-	**Fe**

Table 5: This table indicates the treatments exposed to a concentration of 20 ppm.

Fold Change (2^-ΔΔCT)	Added metal
599.07	**Pb**
66.82	**Cd**
11.5	**Mg**
1.49	**Cu**
-	**Fe**

The results indicate that no growth or germination occurred at high iron concentrations (10 and 20 ppm), which may be attributed to the plant's sensitivity to these levels of the metal or to other contributing factors.

The qPCR-based telomere length measurement in Arabidopsis showed that heavy metal exposure led to increased relative telomeric sequence expression when compared to the control group, The metal-induced stress led to increased telomeric repeats in all treatment conditions which indicates longer telomeres, The observed increase in telomere length may function as a protective mechanism for plant cells to defend chromosome ends from environmental stress

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while preserving genomic stability, The activation of telomerase or other telomere-lengthening mechanisms by living organisms under oxidative or physical stress is supported by previous research, research indicates that prolonged environmental stress may result in telomere shortening after an initial period, The observed telomere elongation during this experiment might represent a short-term cellular reaction to heavy metal exposure, The findings demonstrate the necessity of conducting long-term studies to determine how stressors affect telomere length and its related functions, The research demonstrates that telomere length serves as a significant biomarker for cellular stress while creating opportunities to study plant molecular responses to environmental challenges[19][20][21][22][23. [

CONCLUSIONS

The research demonstrated that heavy metals significantly affect telomere length stability. The environmental stress caused by lead, cadmium and iron exposure leads plants to activate protective mechanisms which result in longer telomeres. The telomere length decreased in plants treated with elevated copper and magnesium concentrations indicating negative effects from these metal levels .

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest with the publication of this work.

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