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STRUCTURE, FUNCTION, AND EVOLUTION OF PLANT TELOMERE COMPONENTS

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Abstract: Telomere plays a crucial role in DNA sequence for protecting genetic information and maintaining chromosomal stability. Many existing studies show the function and structure of telomeres among various species. Despite the increase in analyzing telomeres, the plant telomere components have received little attention and remain largely unexplored. Thus, the components of plant telomeres, as well as the evolutionary views on plant telomere sequence and dysfunction, will be the subject of the current investigation. Fluorescence in situ hybridization (FISH) and quantitative polymerase chain reaction (qPCR) were used to analyse a research sample that was collected from various plant types and habitats. The results demonstrated a strong correlation between sequencing and relative telomere length. In addition, it was shown that telomerase exhibited a notable variation in its mechanism of action when bound close to the 3'-end, and that the oligonucleotide of TEL* had the most significant result.

Keywords: Telomere, Telomerase, Telomere repeat, Replication, Plants, function of Telomere, Plants development, PCR, Fish

1. INTRODUCTION

The terminal terminals of linear chromosomes in eukaryotic organisms are called telomeres. The damages of erroneous recognition and endogenous nucleases can be repaired and preserved by the telomeres, which protect linear chromosomes against the damages as unrepaired chromosomal breaks. Thus, by the telomeric DNA, the structures of the telomeric can be formed [1, 2]. The telomerase enzyme is responsible for repairing the chromosomal ends that have been lost by enzymatic causes. Telomerase is a highly developed mechanism that plays a critical role in preserving the integrity of the genome. [3]. By repetitive DNA, their components of DNA have been usually formed and their replication of conventional machinery can be compensated with specific RNA-dependent DNA polymerase activity [4]. The telomeres may be lengthened for the telomerase reverse transcriptase (TERT) catalysed reverse transcription process by binding telomerase to the single-stranded DNA tail and using short segments containing telomerase RNA (TR) [5, 6]. Figure 1 depicted a diagrammatic illustration of the telomere's structure.

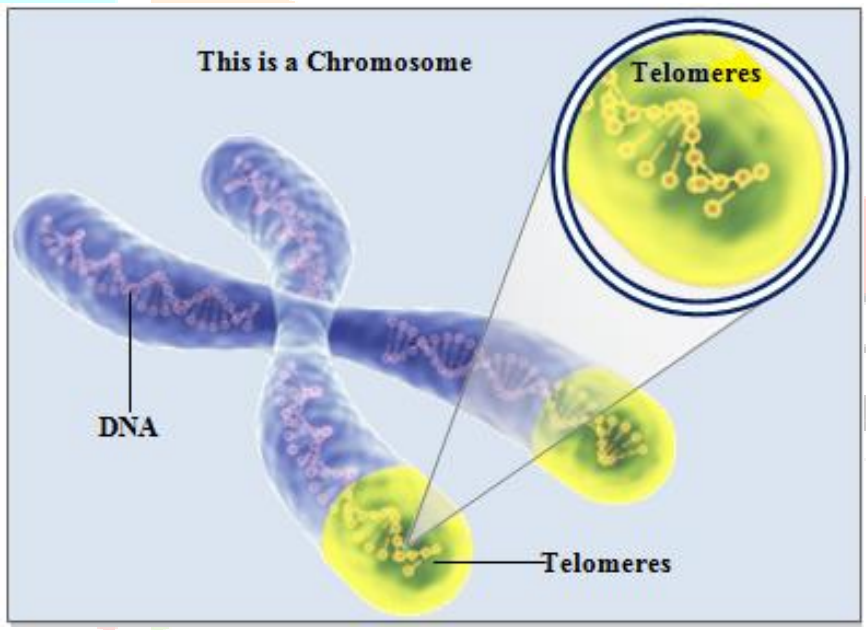

Figure 1: Structure of plant Telomere

The structure of telomeres in plants is very similar to that of mammals. However, compared to animals, plants have fundamentally different development patterns, and this difference may be reflected in the way of telomere synthesis by telomerase, which is regulated. Most significantly, the plants exhibit a highly developmental flexible pattern that is typified by the late germination stage at which plants designate a germ line. A 3' single-stranded DNA overhang often blunts the eukaryotic telomere, which is not terminated.The telomere repeat sequence is TTAGGG in the majority of vertebrates [7, 8]. Unique repeat 7-bp nucleotide units (CCCATTT at the 5 ends and TTTAGGG at the 3 ends) constitute the sequences of plant telomeres, which are highly conserved. In contrast to the adult process, when all organs and tissues are generated from proliferating meristem cells, the plant's body plan was not completely defined during embryogenesis [9, 10]. Thus, the

purpose of this research was to examine how plants adapt to and survive in various habitats by dissecting telomeres and their components. The study's overarching goal is to learn more about the effects of telomere sequence length on plant population dysfunction and to look into the evolutionary viewpoints on plant telomere sequence.

The structure of the paper is enlisted as follows: in Section 2 we have the current literature review, in Section 3 we have the materials and methods, in Section 4 we have the results and discussion of the analysis, and in Section 5 we have the paper's conclusion with future scope.

2. MATERIALS AND METHODS

1.Sample cellection

2.1 The presented study examined the structure-function of plant telomere components. Ideas are sources in environment studies appear are every where plant tree are animal physical parts.

2.2 The research also found the sequence, length, and dysfunction of telomeres in plant populations and looked at the components of telomeres from a perspective of evolution.

2.3 Here, the plant sample has been collected from various plant species and environments.

2.4 Sampling locations will include different geographic regions and ecosystems, with a focus on obtaining a diverse range of plants.

2.5 By applying qPCR,FISH technique, the length, and structure of telomere have been measured. Then, to identify the sequence variations across the different plant species, the bioinformatics analysis of telomere-related genes has been used.

2.6Through the different assays of RT-qPCR and TRAP assay, the collected plant sample of telomere maintenance mechanisms has been investigated. Additionally, we will assess telomerase sequence activity in the plant samples using a TRAP assay. This assay will involve amplifying telomeric repeat sequences using PCR and measuring telomerase activity.

2.7 Telomere Dynamics Analysis involves monitoring the changes in telomere length during different developmental stages of the plant. Thus, to monitor the telomere length changes, qPCR has been used.

Length and dysfunction of plant telomere analysis

In plants, telomere length and dysfunction have been linked to growth abnormalities, reduced reproductive success, and increased susceptibility to environmental stresses. In this study, to analyze telomere dysfunction, various methods have been used, such as Southern blotting, FISH, and telomere-specific PCR.By examining telomere length, telomere structure, and telomerase activity in different plant tissues and under different conditions, we can identify any different changes in plants.

Sequence of Telomere

At the end of DNA strands, the chromosomes are situated in thread-like structures, which contain all genetic information. During cell division, the telomeres become shorter and can reach a point where they are no longer able to protect the chromosomes, which leads to aging and disease development. The distribution of telomere-specific repeats was screened using the plant genome as input. Four possible DNA sequences are known to include the telomeric repeat TTAGGG: TTAGGG (direct), GGGATT (reverse), AATCC (complement), and CCCTAA (reverse complement). A diagrammatic representation of the sequence of telomeres was shown in Figure 2,

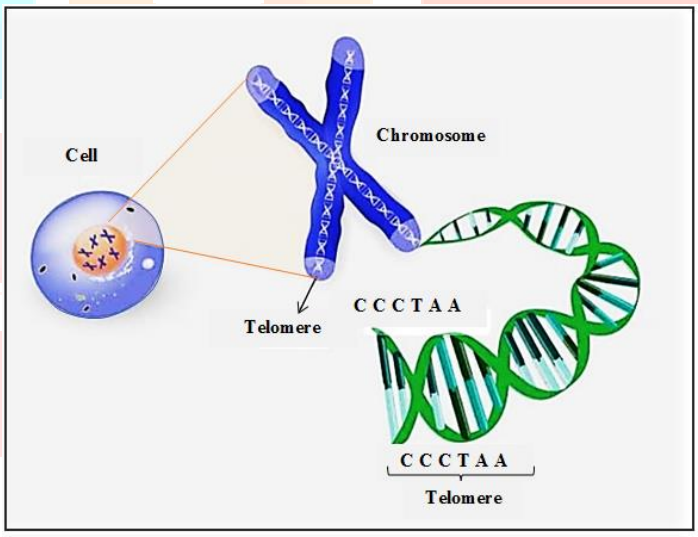

Figure 2: Telomere repeat sequence

2.1. Variation of Telomere length and sequence of observation

Telomere length and sequence variation may provide light on the biology of many species. The replicative capability of cells, the ageing process, and the binding of telomere-associated proteins are all impacted by telomere length and sequence.

Table 1: Analys of telomere length variation and sequence

Telomere length (kb)	Organism	Repeat sequence	Lifespan
2–9	Arabidopsis thaliana	TTTAGGG	Annual
0.5–3.5	Physcomitrella patens	TTTAGGG	NA
1–5.5	Selaginellamoellendorffii	TTTAGGG	Perennial
0.5–30	Pinuspalustris	TTTAGGG	Perennial[1]
2–25	Pinuslongaeva	TTTAGGG	Perennial[2]
2–40	Zea mays	TTTAGGG	Annual
10–50	Pisumsativum	TTTAGGG	Annual
5–11	Oryza sativa	TTTAGGG	Annual
40–160	Nicotianatabacum	TTTAGGG	Annual
>10	Othocallissiberica	TTAGGG*	Biennial

The analysys of variation in telomere length and sequence of observation was given in Table 1 [18]. The various organisms of Arabidopsis thaliana, Physcomitrella patens, Selaginellamoellendorffii, Pinuspalustris, Pinuslongaeva, Zea mays, Pisumsativum, Oryza sativa, Nicotianatabacum, and Othocallissiberica were taken for the analysis. A telomere length greater than 10 indicates a recent transition in the species, as it shows that it has both TTTAGGG and TTAGGG repeats. The "Short-lived" perennial plants (species of superscript 1) were identified as having an average lifetime of 100–200 years. A perennial with a superscript 2 indicates that it is a "long-lived" species, meaning that it typically lives between 2000 and 5000 years. Here, the majority of the selected organisms achieved the annual lifespan with a repeat sequence of TTTAGGG.

2.2. Analysis of Oligos sequence activity of plant telomere using qPCR

This research makes use of oligonucleotides (oligos) that are unique to plant telomere sequences; these oligos include TERT, telomere, PP2A, and RPII, both forward and reverse.

Table 2: Reverse sequence of plant telomere of Oligos

Oligo name	Oligo Sequence (5′–3′)
TERT Forward	GCATCAGAGAAGGGTCAGATT
TERT reverse	CTCTGGCTCCTTGAATCGTATAG
Telomere forward	CCCCGGTTTTGGGTTTTGGGTTTTGGGTTTTGGGT
Telomere reverse	GGGGCCCTAATCCCTAATCCCTAATCCCTAATCCCT
PP2A Forward	CGGCGGCGGGCGGCGCGGGCAGGATAGACATTGGAGGGTTCGGCTCGCAA
PP2A Reverse	CGGCGGCGGGCGGCGCGGGACCACTGCATGCAAAGGGACCCAAGCTTAT
RPII Forward	TGAAGCATACACCTATGATGATGAAG
RPII Reverse	CTTTGACAGCACCAGTAGATTCC

In Table 2, the sequence of plant telomere activity using qPCR was shown [19]. Using the qPCR method, we investigated the relationship between ontogenetic age and relative telomere length as well as TERT expression in plants. Here, the forward and reverse sequence of the telomere, TERT, PP2 A, and RPII have been considered in this study. At a temperature range of 74 to 85◦C, the melting of curve analysis has been performed to ensure no non-specific amplifications for both primer pairs. The telomere and PP2A amplicons then produced the following cycles: 95 ◦C for 30 seconds, 59 ◦C for 1 minute, 72 ◦C for 30 seconds, 84 ◦C for 15 seconds, and 85 ◦C for 15 seconds.

3. RESULT AND DISCUSSION

The section's investigation and discussion focused on two important facets of plant telomeres: the binding of telomere oligonucleotide sequences and the dysfunction found in tert mutants. The samples that were obtained were carefully inspected, and the findings were presented and debated in great detail.

3.1. Binding of Telomere oligonucleotide based sequence

The binding of different telomere oligonucleotide sequences, as measured by their relative binding, is compared to the TEL sequence. The TEL* sequence showed a significantly higher binding affinity compared to the TEL sequence. The binding affinity increased further with the addition of 10 adenine nucleotides (TEL*-a10) or a single guanine nucleotide (TEL*-GG) to the 3' end of the sequence. The addition of three or more guanine nucleotides (TEL*-GGG, TEL*-GTGGG, TEL*-GTGTGGG, and TEL*-GTGTGTGGG) to the 3' end of the sequence resulted in a decrease in binding affinity.

Table 3: Results of binding of TEL and TEL* sequences

Oligonucleotide	Sequence	$k_{d,app}{}^{a}$ (nM)	Relative k_d^b
TEL	GTGTGGGTGTG	212±0.8	-
TEL*	GTGTcTGTcTG	624±123	225
TEL* -a_{10}	GTGTcTGTcTGaaaaaaaaaa	388±67	143
TEL* -GG	GTGTcTGTcTGGG	129±23	52
TEL* -GGG	GTGTcTGTcTGGGG	41±5.13	17
TEL* -GTGGG	GTGTcTGTcTGGTGGG	16±1.7	8
TEL* -GTGTGGG	GTGTcTGTcTGGTGTGGG	31±4.9	8.15
TEL* -GTGTGTGGG	GTGTcTGTcTGGTGTGTGGG	17±1.14	4.8

The binding of oligonucleotide TEL and TEL* sequences is shown in Table 3 [20]. The kd numbers shown here are the mean standard errors of three separate apparent kd measurements. The kd of the TEL oligonucleotide is used to display the bkd values. The oligonucleotide of TEL* achieved the highest mean value, which is 624±123, and its relative $^{b}k_{d}$is 225. Then, the oligonucleotide of TEL* -a_{10} obtained the second highest mean value i.e. 388±67, and its relative $^{b}k_{d}$is 143. Thereafter, the third highest mean value is achieved by TEL 212±0.8, while, TEL* -GTGGG obtained the lowest mean value, which is 16±1.7, and its relative $^{b}k_{d}$is 8.

3.2. Dysfunction of plant telomere

Low quantities of chromosomal fusions were seen during the anaphase of briding in mitotic cells in late-generation plants, but mutant phenotypes often emerged in early-generation plants [21]. Here, the genotype of early (tertG2), late (tertG7), and Wild-Type (WT) has been analyzed. Mutants allow for the separation of effects of telomerase enzyme lack from the sequence of genomic damage and uncapped telomeres. Figure 3 depicted the process of creating tert mutant plants graphically.

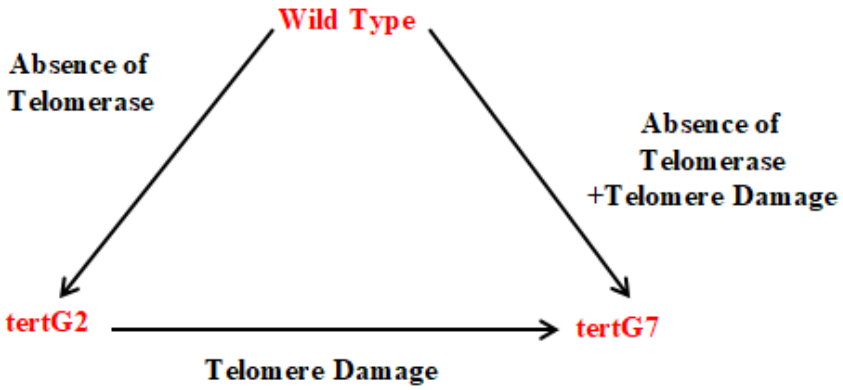

Figure 3: Generation of Tert mutants

Table 4 represents the analysis of the generation of Tert mutants with the genotype of germination of seeds and mitoses with anaphase bridges.

Table 4: Results of generation of TERT mutants

Genotype	**WT**	**tertG2**	**tertG7**
Germination of seeds	97%	95%	67%
% of mitoses with anaphase bridges	0.11%	0%	38.5%

The results of the experiment are provided in Table 4, which shows that the tertG7 plants had poor growth and seed germination, which increased cell death and retarded mitosis. 38.5% of mitoses with anaphase bridges show a mildness of the impact of plant and remain able to develop. Two or three further generations of tert plants are much more seriously impacted, since they are unable to grow or shrink (tert G9–11). Thus, figure 4 shows the mean of M-phase of genotype.

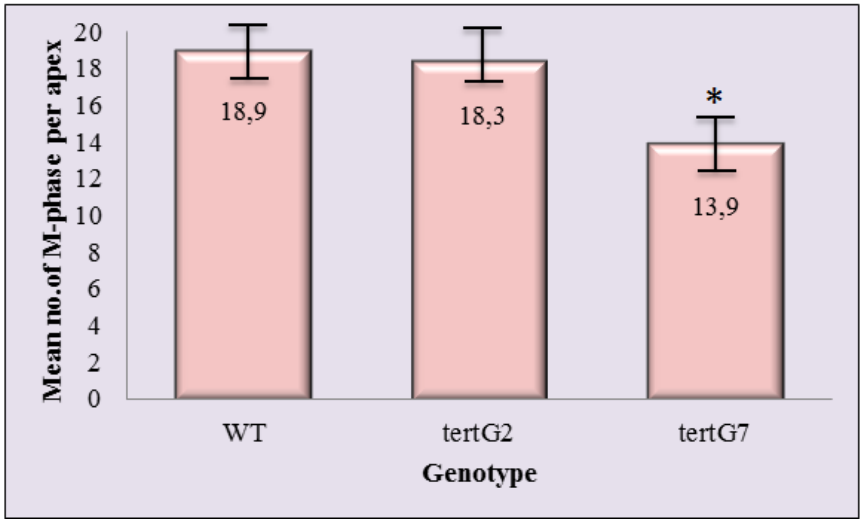

Figure 4: Regulation of root tips of WT, tertG2, and tertG7 mutants

Figure 4 shows the average amount of mitotic nuclei in the M-phase per root tip of WT, tertG2, and tertG7 seedlings. Therefore, the asterisk indicates a statistically significant difference between the WT and tertG7 mutants, while the error bars depict the standard errors. They obtained the mean of the M-phase of WT (18,9), tertG2 (18,3), and tertG7 (13,9).

4. CONCLUSION

The purpose of this research was to examine the role and structure of telomeres in plants. Further, this study explored the length and sequence of telomeres and dysfunction of plants. A sample has been considered from various plant species. The study's results were examined utilising q-PCR and FISH methods. This concluded that the oligonucleotide of TEL* achieved the highest mean value, which is 624±123, and its relative $^{b}k_{d}$ is 225. Thereafter, the majority of plant organisms achieved the annual lifespan with a repeat sequence of TTTAGGG. Additionally, it was shown that when telomerase bound at the 3'-end, there were notable changes in the mechanisms involving the telomere end binding protein. Finally, the tertG7 (67%) obtained poor development compared with WT and tertG2. However, in this study, a study sample has been considered with limited plant species. In the future, this can be considering more plant species and exploring the developmental activity of plant telomere and their significance level. This study leads hopes to gain insights into their biological functions and potential applications in agriculture and medicine. The findings from this research might be used in plant breeding and genetic engineering to create types of plants that are more resistant to certain stresses.

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