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Structure, Function, and Benefits of Chloroplast DNA: Review Article

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ABSTRACT

Chloroplasts are double-membrane organelles that contain extranuclear DNA. The existence of this chloroplast genome (cpDNA) has an essential value for plant survival. A review of cpDNA is necessary because it underpins photosynthesis and plant acclimation, which directly affect plant productivity and ecosystem stability. In addition, cpDNA is increasingly applied in DNA barcoding and chloroplast engineering, yet the key concepts are often scattered across genetics, physiology, and biotechnology, making an integrated synthesis valuable. The method and analysis used are articles reviewed by searching, collecting, and analyzing related research articles. The main results obtained are the chloroplast genome in the form of a single quadripartite circular, its primary function for the synthesis of photosynthetic enzymes, and other functions as the biosynthesis of macromolecular compounds and secondary chloroplast metabolites, and plays a role in response to environmental stress, while the replication mechanism follows the maternal pattern in most Angiosperms and paternal in most Gymnosperms. The benefits of the chloroplast genome itself include the manufacture of recombinant pharmaceutical proteins, DNA barcoding, and Transplastomic. These results indicate that cpDNA has a crucial role in the survival of plants and humans. Further research and review regarding the relationship between environmental variables and the chloroplast genome are needed to complete the discussion on cpDNA.

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1. INTRODUCTION

Chloroplasts are endosymbiotic organelles that have a double membrane where photosynthesis takes place (Maulid et al., 2014). The circular chloroplast genome (cpDNA) contains around 120-130 genes involved in energy production and gene expression (Martin et al., 2013; Daniell et al., 2016). Chloroplasts produce energy through the process of photosynthesis, which supports plant growth, so chloroplasts are responsible for the biosynthesis of active compounds such as amino acids, phytohormones, nucleotides, vitamins, lipids, and secondary metabolites. Other functions are related to energy metabolism, transcription, and translation (Song et al., 2021).

In addition to functioning as an energy producer and biosynthesis, the chloroplast genome (cpDNA) has an important value in plant responses to temperature stress (Wang et al., 2018), salinity stress (Ling & Jarvis, 2015), and drought stress (Wannarat et al., 2017). Furthermore, the importance of chloroplasts can be viewed from its benefits to the advancement of science, namely in the fields of agriculture, health, and taxonomy. The unique replication and transcription-translation mechanisms also add to this important value.

The large role and importance of chloroplasts, especially the chloroplast genome (cpDNA), make it interesting to review. However, information on how the chloroplast mechanism or chloroplast genome copes with environmental stress is not widely known. In addition, information on the structure, replication mechanism, and transcription-translation mechanism has not been fully discussed.

Several studies have revealed that chloroplast responses to high-temperature stress involve complex pathways, including increased activities of chlorophyll-degrading enzymes (Rossi et al., 2017) and elevated production of reactive oxygen species (ROS) (Vacca et al., 2016). Beyond stress physiology, cpDNA also provides practical benefits in the medical field through chloroplast genetic engineering for recombinant pharmaceutical protein production, including monoclonal antibodies (Rajabi-Memari et al., 2006), antibiotic proteins (Oey et al., 2009), interferon alpha (Arlen et al., 2007), and interferon beta (Feizi & Baghbankohnehrouz, 2021). Therefore, this review compiles recent knowledge on chloroplast DNA by summarizing its structure, functions, gene expression and inheritance mechanisms, and key benefits. By presenting these aspects in an integrated manner, this article aims to serve as a reference and to address central questions regarding chloroplast DNA (cpDNA).

2. RESEARCH METHOD

This literature review was conducted by searching, collecting, and synthesizing peer-reviewed articles from national and internationally accredited journals accessed through Google Scholar, PLOS ONE, Elsevier, and ScienceDirect (with additional retrieval through ResearchGate when necessary). Search keywords related to chloroplast DNA included: chloroplast DNA characteristics, chloroplast genome structure, cpDNA recombination, cpDNA inheritance, chloroplast DNA transcription (PEP/NEP), cpDNA replication, DNA coding, chloroplast structure and function, and essential chloroplast genes. The selected literature was analyzed to answer the article title by summarizing cpDNA structure (genome organization and gene content), function (role in photosynthesis and plant metabolism/stress response), and benefits (applications in agriculture, health biotechnology, and taxonomy).

3. RESULTS AND ANALYSIS

3.1. Chloroplast DNA Structure

Chloroplasts have their circular genome and play a crucial role in photosynthesis, physiology, and development in most plants. The chloroplast DNA molecules are typically distributed within the chloroplast stroma (Triani, 2021). Compared to the nuclear genome, the chloroplast genome is more conserved in terms of size, gene content, genomic structure, and the linear sequence of genes (Zhu et al., 2020). Chloroplast DNA (cpDNA) consists of a single circular molecule with a quadripartite structure, which is divided into four main segments or regions. These include two copies of the inverted repeat (IR) region separated by the large single-copy (LSC) and small single-copy (SSC) regions (Martin et al., 2013). The size of the chloroplast genome varies among species, typically ranging from 120 to 170 kb (Jong-Hwa et al., 2017). The chloroplast genome contains 120–130 genes, primarily involved in photosynthesis, transcription, and translation (Daniell et al., 2016). Most of these genes are organized into operons (or operon-like structures). They are transcribed as precursor polycistronic molecules, which undergo splicing and nucleolytic cleavage to produce mature mRNA that can be translated (Wicke et al., 2011). In general, the chloroplast genome (cpDNA) includes the 16S, 23S, and 5S rRNA genes and tRNA genes (Zhang et al., 2016), which are sufficient for translating all amino acids and at least three of the four subunits of the prokaryotic-type RNA polymerase (rpoB, C1, C2). Additionally, most genes are present in operon form for polypeptides involved in Photosystem I, Photosystem II, the cytochrome b6f complex, and ATP synthase (Green,

2011). The sequence and expression mechanisms of genes in these operons are very similar to those found in prokaryotes, with the main structural difference between chloroplast genes and prokaryotic genes being the presence of introns (Palmer et al., 2012).

The well-conserved chloroplast genome (Zhu et al., 2020) is commonly used for phylogenetic analysis at higher taxonomic levels (family level). The complete chloroplast genome sequence of plants was first reported in tobacco in 1986. Currently, more than 800 chloroplast genome sequences are available at the National Center for Biotechnology Information (NCBI). One example of research conducted by Jong-Hwa et al. (2017), which sequenced the chloroplast genome of Lilium species (**Figure 1**), revealed a total of 156 genes, including 102 protein-coding genes, 46 tRNA genes, and 8 ribosomal RNA (rRNA) genes. The LSC and SSC regions each contain 96 and 12 genes, respectively, and each IR region contains 24 genes oriented in reverse directions. Based on the chloroplast genome map, the genome sequences of *L. amabile*, *L. kalosum*, and *L. lancifolium* are identical but differ from the cp genome of *L. philadelphicum*.

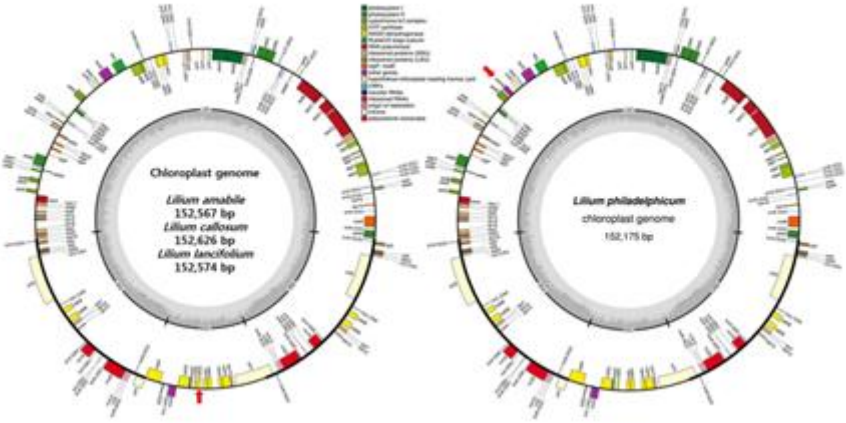

Figure 1. Chloroplast genome map of four Lilium species
(Source: Jong-Hwa et al., 2017)

3.2. Chloroplast DNA Function

In general, the function of chloroplasts is as a place where photosynthesis takes place (Serrano et al., 2016). Chloroplasts produce energy through photosynthesis and oxygen release processes, so chloroplasts are responsible for the biosynthesis of active compounds such as amino acids, phytohormones, nucleotides, vitamins, lipids, and secondary metabolites (Song et al., 2021). Chloroplasts have an important role in plant acclimatisation to environmental stress, where chloroplasts can synthesise biologically active compounds and phytohormones when plants are under adverse environmental conditions.

1. Chloroplast response to temperature stress

Chloroplasts are known to be sensitive to high-temperature stress during the photosynthesis process (Wang et al., 2018). Heat treatment causes chlorophyll-degrading enzymes to increase significantly, while the activity of the main chlorophyll-synthesising enzyme does not change (Rossi et al., 2017). When plants are at high temperatures, large amounts of ROS (Reactive Oxygen Species) are produced to survive (Vacca et al., 2016). According to Martins et al (2016), ROS levels contribute to mitigating PSII photoinhibition in coffee plants. When plants respond to heat stress, chloroplast heat-shock protein (Hsp) 21 is bound to the thylakoid membrane and plays a role in stress resistance.

Low temperature also affects the abundance of proteins involved in photosynthesis (Gan et al., 2019). When exposed to low temperatures, chloroplasts change the content of unsaturated fatty acids in the chloroplast membrane to increase plants' tolerance to temperature (Xue et al., 2019). When adapting to cold temperatures, enzyme activity is regulated in dark reactions, such as the reduction of enzymes involved in the Calvin cycle, fructose-1,6-diphosphatase (FBPase), and isoheptanone-1,7-diphosphatase (SBPase) (Kingston-Smith et al., 1997). Based on research conducted (Shi et al., 2014) using Cynodon dactylon, when plants respond to cold stress, the

photosynthetic electron transport chain in chloroplasts transfers excess electrons to O2 and causes an increase in O2-. Under low-temperature stress, the application of acetylsalicylic acid can increase the activity of the chloroplast antioxidant system, thereby increasing its tolerance to low temperatures (Soliman et al., 2018). According to Wang et al. (2016), the RNA-binding domain RBD1 in chloroplasts affects the low-temperature tolerance of plants in the process of protein translation. RBD1 binds to 23S rRNA and affects 23S rRNA processing in *Arabidopsis* plants.

2. Chloroplast response to salinity stress

When salt stress occurs, plants regulate protein transport to the chloroplast to increase tolerance through increased transcription of the ubiquitin E3 ligase protein suppressor of PPI1 locus 1 (SP1). SpP1 causes degradation of TOC (Translocon at the Outer Envelope Membrane of Chloroplast), inhibits photosynthesis, and decreases ROS formation (Ling & Jarvis, 2015). Pulido et al. (2017) found that Hsp70 protein (chloroplast companion protein) and Clp protease can protect plants from environmental stresses such as salinity stress. In the event of stress, Hsp70 performs protein refolding and Clp degrades proteins that fail to fold and undergo aggregation (Pulido et al., 2017).

3. Chloroplast response to drought

When plants respond to drought, the SAL1-PAP retrograde pathway in chloroplasts regulates stress-responsive genes and stomatal closure to increase drought tolerance (Wannarat et al., 2017). Based on research conducted by Yoo et al. (2017), it is known that phytochrome B (OsPhyB) in rice suppresses ascorbate peroxidase (APX) activity and catalyzes ROS required for drought tolerance. This suggests that OsPhyB in rice has the potential to manipulate drought tolerance.

3.3 Chloroplast DNA Expression and Inheritance Mechanisms

CpDNA genome is inherited maternally in most flowering plants (angiosperms), although in most gymnosperms (conifers and cycads) it is usually inherited paternally. DNA recombination occurs occasionally; chloroplasts are mostly structurally stable and mostly vary in size and thus differ in length and sequence repetition, in contrast to the rearrangement and duplication of genes found in plant mtDNA (mitochondrial DNA). Based on transcriptional control, cpDNA genes can be grouped into three classes (I–III) (Hajdukiewicz et al., 1997). Class I genes are transcribed only by PEP (plastid-encoded RNA polymerase), Class II genes are transcribed by both PEP and NEP (nucleus-encoded RNA polymerase), and Class III genes are transcribed exclusively by NEP. In higher plants, many chloroplast genes are organized in clusters and are initially transcribed as polycistronic precursor RNAs, which are subsequently processed into shorter (often overlapping) RNAs (Sugita & Sugiura, 1996).

The mechanism of chloroplast DNA (cpDNA) replication has not been definitively determined. Scientists have attempted to observe chloroplast replication by electron microscopy since the 1970s. Microscopic experiments have led to the idea that chloroplast DNA replicates using a double displacement loop (D-loop). As the D-loop moves through the circular DNA, it adopts a theta intermediate shape, also known as a Cairns replication intermediate, and completes replication by a rolling circle mechanism. Transcription begins at a specific origin. Many replication forks open, allowing the replication machinery to copy the DNA. As replication continues, the forks grow and eventually fuse. The new cpDNA structures separate, creating cpDNA daughter chromosomes. One competing model for cpDNA replication posits that most cpDNA is linear and participates in homologous recombination and replication structures similar to the linear and circular DNA structures of bacteriophage T4. It has been established that some plants have linear cpDNA, such as maize, and many more species still contain complex structures that scientists do not yet understand. When the original experiments on cpDNA were performed, scientists did observe linear structures; however, they attributed these linear forms to broken circles. If the branched and complex structures seen in cpDNA experiments are real and not artefacts of spliced circular DNA or broken circles, then the D-loop replication mechanism is insufficient to explain how such structures would replicate (Bendich AJ, 2004). At the same time, homologous recombination does not extend some of the A→G gradients seen in plastomes. Because of the failure to explain the deamination gradient as well as the many plant species that have been shown to have circular cpDNA, the dominant theory continues to

be that most cpDNA is circular and most likely replicates via the D-loop mechanism (Krishnan NM & Rao BJ, 2009).

3.4 Benefit of cpDNA

Chloroplast DNA or chloroplast genome has many benefits for human life. These benefits include in the field of agriculture for the creation of superior varieties, in the field of medicine for the creation of drugs, and in the field of taxonomy for classifying certain types of plants.

1. Agriculture

The need for staple foods that increase every year due to the increase in the human population amidst the shrinking agricultural land due to land conversion has motivated researchers to develop various superior plant varieties in terms of productivity and nutrition. This development is carried out massively by engineering nuclear DNA through various methods such as transgenic DNA and DNA editing. However, in addition to engineering nuclear DNA, recently many studies have also led to engineering Chloroplast DNA to create future plants (Transplastomic). Jackson et al. (2021) in his research, for example, stated that the chloroplast genome of the green algae species *Chlamydomonas reinhardtii* can be engineered to change the metabolism of the green algae to produce desired products such as the insertion of nitrogen fixation genes, carbon fixation, terpenoids, and vitamin B12. In addition, other research conducted by Gangl et al. (2015) conducted the insertion of the CYP79A1 gene encoding the cytochrome P450 enzyme by the transformation method in *Chlamydomonas* chloroplasts, which produced stable cytochrome P450 enzymes and a reaction pathway for the synthesis of diterpenoid compounds. Although the research is still in its early stages in lower organisms that are close to the plant group, the application of chloroplast DNA engineering in plants is expected to overcome nutritional problems and the production of necessities.

2. Health sector

To produce recombinant proteins (especially pharmaceutical proteins) in large quantities, plants are a suitable alternative compared to mammalian species, insects, yeast cultures, or microbial bioreactors (Feizi & Baghbankohnehrouz, 2021). This is because, compared to other systems, the production of recombinant pharmaceutical proteins through chloroplast genome engineering has advantages such as high accumulation of recombinant proteins due to natural polyploidy, limited number of protein degradation pathways, small side effects, and silencing due to directional integration of homologous transgenes in specific regions of the chloroplast genome and maternal chloroplast gene inheritance; economically competitive; easily scalable; and capable of carrying the complex post-translational modifications required for recombinant pharmaceutical proteins (Abd-Aziz et al., 2020). Meanwhile, compared to using the chloroplast nuclear genome, it offers several advantages such as high expression levels, the occurrence of position effects, low risk of transgene spread to the environment, the ability to express polycistronic genes, and clear gene silencing (Karimi et al., 2013 in Feizi & Baghbankohnehrouz, 2021). Pharmaceutical recombinant proteins that have been successfully developed using chloroplast genome engineering are monoclonal antibodies (Rajabi-Memari et al., 2006), antibiotic proteins (Oey et al., 2009), Interferon alpha (Arlen et al., 2007), and interferon beta (Feizi & Baghbankohnehrouz, 2021).

3. Taxonomy

Accurate, reliable, and efficient plant identification is essential for flora richness and diversity conservation, assessment, and land-use regulation (Nevill et al., 2020). Standard morphology-based classification can be unreliable for large and complex taxa (Liu et al., 2017), when only fragments are available (Jones et al., 2011), and at certain life stages (Gonzalez et al., 2009). DNA barcoding therefore complements morphology by enabling reproducible identification from small tissue samples using standardized loci (Liu et al., 2017). In land plants, commonly used barcoding regions are largely chloroplast-derived (e.g., rbcL, matK, trnH–psbA, trnL–F, rps16, rpl16) and are sometimes combined with nuclear markers such as ITS (Azani et al., 2017). As a community standard, the CBOL Plant Working Group recommended the two-locus chloroplast barcode rbcL + matK based on recoverability, sequence quality, and species discrimination (CBOL Plant Working Group, 2009). Quantitatively, comparative testing across diverse genera showed that no single locus discriminated species pairs in >79% of genera, while discrimination increased to ~88% when trnH–psbA was paired with a coding locus such as rbcL; moreover, GenBank data-mining tests indicated that rbcL + trnH–psbA produced 95.0% correct species-level matches (Kress & Erickson, 2007).

More recent next-generation barcoding further improves accuracy by using plastome-scale data (super-barcodes): in the taxonomically challenging genus Acer, whole plastomes achieved 90.47% species discriminatory power compared with 61.90% for the standard combination matK + rbcL + trnH–psbA (Fu et al., 2024). However, plastid-based identification can still fail in lineages shaped by hybridization because incomplete lineage sorting and chloroplast capture may obscure species boundaries; integrating nuclear data (e.g., nrDNA) can help for such cases (Fu et al., 2024). These barcode datasets can subsequently be extended into phylogenetic reconstruction to infer relationships among taxa (Pang et al., 2019).

4. CONCLUSION

Based on a literature review of the structure, function, and benefits of chloroplast DNA (cpDNA), several conclusions can be drawn. Chloroplast DNA is generally a single circular molecule containing approximately 120–130 genes and exhibits a conserved quadripartite organization, consisting of two inverted repeat (IR) regions separated by a large single-copy region (LSC) and a small single-copy region (SSC). Many chloroplast genes are organized in operon-like clusters and are initially transcribed as polycistronic precursor RNAs that are processed into mature transcripts, with introns representing a major structural difference from typical prokaryotic gene organization. Functionally, chloroplasts serve as the primary site of photosynthesis and also contribute to plant responses to environmental stresses, including temperature, salinity, and drought. In terms of inheritance and maintenance, cpDNA is inherited maternally in most angiosperms and more commonly paternally in many gymnosperms, while the displacement-loop (D-loop) model remains the widely accepted explanation for cpDNA replication. Finally, cpDNA offers broad benefits to humans through chloroplast genome engineering to develop improved crop varieties, production of recombinant pharmaceutical proteins, and its use in taxonomy through DNA barcoding and phylogenetic reconstruction for plant identification and classification..

REFERENCES

Abd-Aziz, N., Tan, B. C., Rejab, N. A., Othman, R. Y., & Khalid, N. (2020). A new plant expression system for producing pharmaceutical proteins. *Molecular Biotechnology, 62,* 240-251.

Arlen, P. A., Falconer, R., Cherukumilli, S., Cole, A., Cole, A. M., Oishi, K. K., & Daniell, H. (2007). Field production and functional evaluation of chloroplast-derived interferon-α2b. *Plant biotechnology journal, 5(4)*, 511-525.

Azani, N., Babineau, M., Bailey, C.D., Banks, H., Barbosa, A.R., Pinto, R.B., Boatwright, J.S., Borges, L.M., Brown, G.K., Bruneau, A. and Candido, E. (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny: The Legume Phylogeny Working Group (LPWG). *taxon, 66(1)*, pp.44-77.

Bendich AJ, (2004). Circular chloroplast chromosomes: the grand illusion. *The Plant Cell. 16 (7)*: 1661–6. doi:10.1105/tpc.160771. PMC 514151. PMID 15235123.

CBOL Plant Working Group. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America, 106*(31), 12794–12797. https://doi.org/10.1073/pnas.0905845106

Daniell, H., Lin, C. S., Yu, M., and Chang, W. J. (2016). Chloroplast genomes: Diversity, evolution, and applications in genetic engineering. *Genome Biology, 17*(1),1–29, DOI: https://doi.org/10.1186/s13059-016-1004-2.

Feizi, A. and Baghbankohnehrouz, B. (2021) .Overexpression of the recombinant human interferon-beta (rhIFN-β) gene in tobacco chloroplasts. *BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology*, 102(4),367–376, DOI:10.5114/BTA.2021.111094.

Fu, N., Xu, Y., Jin, L., Xiao, T.-W., Song, F., Yan, H.-F., Chen, Y.-S., & Ge, X.-J. (2024). Testing plastomes and nuclear ribosomal DNA sequences as the next-generation DNA barcodes for species identification and phylogenetic analysis in *Acer*. *BMC Plant Biology, 24*, 445. https://doi.org/10.1186/s12870-024-05073-w

Gan, P., Liu, F., Li, R., Wang, S. and Luo, J. (2019). Chloroplasts— Beyond Energy Capture and Carbon Fixation: Tuning of Photosynthesis in Response to Chilling Stress. *International Journal of Molecular Sciences*, 20(20), p.5046, DOI:10.3390/ijms20205046.

Gangl, D., Zedler, J.A.Z., Włodarczyk, A., Jensen, P.E., Purton, S. and Robinson, C. (2015). Expression and Membrane-Targeting of an Active Plant Cytochrome P450 in The Chloroplast of The Green Alga Chlamydomonas reinhardtii. *Phytochemistry*, 110, 22–28, DOI:10.1016/j.phytochem.2014.12.006.

Gonzalez, M.A., Baraloto, C., Engel, J., Mori, S.A., Pétronelli, P., Riéra, B., Roger, A., Thébaud, C. and Chave, J. (2009). Identification of Amazonian trees with DNA barcodes. *PLoS one, 4(10)*, p.e7483.

Green, B.R .(2011).Chloroplast genomes of photosynthetic eukaryotes. *Plant Journal, 66*(1), pp. 34–44, DOI: 10.1111/j.1365-313X.2011.04541.x.

Hajdukiewicz PRJ, Allison LA, Maliga P. (1997). The two RNA polymerase encoded by the nuclear and the plastid compartments transcribe distinct groups of genes in tobacco plastids. *EMBO J,* 4041-4048, DOI:10.1093/emboj/16.13.4041

Jackson HO, Taunt HN, Mordaka PM, Smith AG, Purton S .(2021). The Algal Chloroplast as a Testbed for Synthetic Biology Designs Aimed at Radically Rewiring Plant Metabolism. *Front Plant Sci*, DOI: 10.3389/fpls.2021.708370.

Jones, F.A., Erickson, D.L., Bernal, M.A., Bermingham, E., Kress, W.J., Herre, E.A., Muller-Landau, H.C. and Turner, B.L. (2011). The roots of diversity: below ground species richness and rooting distributions in a tropical forest revealed by DNA barcodes and inverse modeling. *PLoS One, 6(9)*, p.e24506.

Jong-Hwa, K., Sung-Il, L., Bo-Ram, K., Ik-Young, C., Peter, R., and Nam-Soo, K. (2017). Chloroplast genomes of Lilium lancifolium, L. amabile, L. callosum, and L. philadelphicum: Molecular characterization and their use in phylogenetic analysis in the genus Lilium and other allied genera in the order Liliales. *PLoS ONE, 12*(10), pp. 1–15, DOI:10.1371/journal.pone.0186788

Kingston-Smith, A.H., Harbinson, J., Williams, J. and Foyer, C.H. (1997). Effect of Chilling on Carbon Assimilation, Enzyme Activation, and Photosynthetic Electron Transport in the Absence of Photoinhibition in Maize Leaves. *Plant Physiology*, 114(3), 1039–1046, DOI:10.1104/pp.114.3.1039.

Kress, W. J., & Erickson, D. L. (2007). A two-locus global DNA barcode for land plants: The coding *rbcL* gene complements the non-coding *trnH–psbA* spacer region. *PLOS ONE, 2*(6), e508. https://doi.org/10.1371/journal.pone.0000508

Krishnan NM, Rao BJ. (2009). A comparative approach to elucidate chloroplast genome replication. BMC Genomics. 10 (237): 237. doi:10.1186/1471-2164-10-237. PMC 2695485. PMID 19457260.

Ling, Q. and Jarvis, P. (2015). Regulation of Chloroplast Protein Import by the Ubiquitin E3 Ligase SP1 is Important for Stress Tolerance in Plants. *Current Biology*, 25(19), 2527–2534. DOI:10.1016/j.cub.2015.08.015.

Liu, Z. F., Ci, X. Q., Li, L., Li, H. W., Conran, J. G., & Li, J. (2017). DNA barcoding evaluation and implications for phylogenetic relationships in Lauraceae from China. *PloS ONE, 12*(4), e0175788, DOI:10.1371/journal.pone.0175788.

Maghfiroh, K. (2017). Identification of Chlorophyll Content in Genus Piper (Sirih) as Food Supplement Candidate. *Teknologi Pangan: Media Informasi Dan Komunikasi Ilmiah Teknologi Pertanian 8 (1),* 93-98, DOI:10.35891/tp.v8i1.540

Martins, M.Q., Rodrigues, W.P., Fortunato, A.S., Leitão, A.E., Rodrigues, A.P., Pais, I.P., Martins, L.D., Silva, M.J., Reboredo, F.H., Partelli, F.L., Campostrini, E., Tomaz, M.A., Scotti-Campos, P., Ribeiro-Barros, A.I., Lidon, F.J.C., DaMatta, F.M. and Ramalho, J. . (2016). Protective Response Mechanisms to Heat Stress in Interaction with High [CO2] Conditions in *Coffea* spp. *Frontiers in Plant Science*, 7, DOI:10.3389/fpls.2016.00947.

Martin, G., Baurens, F.C., Cardi, C., Aury, J.M. and D'hont, A. (2013). The complete chloroplast genome of banana (Musa acuminata, Zingiberales): insight into plastid monocotyledon evolution. *PloS one, 8(6)*, e67350.

Maulid, R., R., Laily, A., R. (2015). Kadar total pigmen klorofil dan senyawa antosianin ekstrak kastuba (Euphorbia pulcherrima) berdasarkan umur daun. *Prosiding* KPSDA 1 (1).

Nevill, P.G., Zhong, X., Tonti-Filippini, J., Byrne, M., Hislop, M., Thiele, K., Van Leeuwen, S., Boykin, L.M. and Small, I. (2020). Large scale genome skimming from herbarium material for accurate plant identification and phylogenomics. *Plant Methods, 16*, 1-8.

Oey, M., Lohse, M., Kreikemeyer, B. and Bock, R. (2009). Exhaustion of the chloroplast protein synthesis capacity by massive expression of a highly stable protein antibiotic. *The plant journal*, 57(3), pp.436-445, DOI:10.1111/j.1365-313X.2008.03702.x

Palmer, J. D., Jansen, R. K., Michaels, H. J., Chase, M. W., James, R., Palmer, D., and Manhart, J. R . (2012). Variation and Plant Phylogeny1, *Garden, 75*(4), 1180–1206.

Pang, X., Liu, H., Wu, S., Yuan, Y., Li, H., Dong, J., Liu, Z., An, C., Su, Z. and Li, B. (2019). Species identification of oaks (Quercus L., Fagaceae) from gene to genome. *International journal of molecular sciences, 20(23)*, p.5940.

Pulido, P., Llamas, E. and Rodriguez-Concepcion, M. (2017). Both Hsp70 Chaperone and Clp Protease Plastidial Systems are Required for Protection Against Oxidative Stress. *Plant Signaling & Behavior*, 12(3), p.e1290039, DOI:10.1080/15592324.2017.1290039.

Rajabi-Memari, H., Jalali-Javaran, M., Rasaee, M.J., Rahbari Zadeh, F., Forouzandeh-Moghadam, M. and Esmaili, A. (2006). Expression and characterization of a recombinant single-domain monoclonal antibody against MUC1 mucin in tobacco plants. *Hybridoma*, 25(4), pp.209-215, DOI:10.1089/hyb.2006.25.209

Rossi, S., Burgess, P., Jespersen, D. and Huang, B. (2017). Heat-Induced Leaf Senescence Associated with Chlorophyll Metabolism in Bentgrass Lines Differing in Heat Tolerance. *Crop Science*, 57(S1), p.S-169-S-178, DOI:10.2135/cropsci2016.06.0542.

Serrano, I., Audran, C. and Rivas, S. (2016).Chloroplasts at work during plant innate immunity. *Journal of Experimental Botany*, 67(13), pp.3845–3854, DOI:10.1093/jxb/erw088.

Shi, H., Jiang, C., Ye, T., Tan, D., Reiter, R.J., Zhang, H., Liu, R. and Chan, Z. (2014). Comparative Physiological, Metabolomic, and Transcriptomic Analyses Reveal Mechanisms of Improved Abiotic Stress Resistance in Bermudagrass [*Cynodon dactylon* (L). Pers.] by exogenous melatonin. *Journal of Experimental Botany*, 66(3),681–694, DOI:10.1093/jxb/eru373.

Soliman, M.H., Alayafi, A.A.M., El Kelish, A.A. and Abu-Elsaoud, A.M. (2018). Acetylsalicylic Acid Enhance Tolerance of Phaseolus Vulgaris L. to Chilling Stress, Improving Photosynthesis, Antioxidants and Expression of Cold Stress Responsive Genes. *Botanical Studies*, 59(1), DOI:10.1186/s40529-018-0222-1.

Song, Y., Feng, L., Alyafei, M.A.M., Jaleel, A. and Ren, M. (2021). Function of Chloroplasts in Plant Stress Responses. *International Journal of Molecular Sciences*, 22(24),13464. DOI:10.3390/ijms222413464.

Sugita M, Sugiura M. (1996). Regulation of gene expression in chloroplasts of higher Plants. *Plant Molecular Biology*, 32:315-326, DOI:10.1007/BF00039388.

Triani, N. (2021). Aplikasi Penanda Molekuler cpSSR untuk Tanaman Jeruk Hasil Fusi Protoplas. *Prosiding Seminar Nasional Agroteknologi*, 978–623.

Vacca, R.A., Valenti, D., Bobba, A., Merafina, R.S., Passarella, S. and Marra, E. (2006). Cytochrome c Is Released in a Reactive Oxygen Species-Dependent Manner and Is Degraded via Caspase-Like Proteases in Tobacco Bright-Yellow 2 Cells en Route to Heat Shock-Induced Cell Death. *Plant Physiology*, 141(1), 208–219. DOI:10.1104/pp.106.078683.

Wannarat, P., Estavillo, G.M., Chan, K.X., Tee, E.E., Diep, G., Crisp, P.A., Yin, P.S., Chenchen, Z., Jiaen, Q., Park, J., Tiem, Y.M., Nazia, N., Yadav, A.K., Benjamin, S., Rathjen, J., Cazzonelli, C.I., Wilson, P.B., Matthew, G., Zhong-Hua, C. and Pogson, B.J. (2017). A Chloroplast Retrograde Signal, 3'-Phosphoadenosine 5'-Phosphate, Acts as a Secondary Messenger in Abscisic Acid Signaling in Stomatal Closure and Germination. *ProQuest*, DOI:10.7554/eLife.23361.

Wang, S., Bai, G., Wang, S., Yang, L., Yang, F., Wang, Y., Zhu, J.-K. and Hua, J. (2016). Chloroplast RNA-Binding Protein RBD1 Promotes Chilling Tolerance through 23S rRNA Processing in Arabidopsis. *PLOS Genetics*, 12(5), p.e1006027, DOI:10.1371/journal.pgen.1006027.

Wang, Q.-L., Chen, J.-H., He, N.-Y. and Guo, F.-Q. (2018). Metabolic Reprogramming in Chloroplasts under Heat Stress in Plants. *International Journal of Molecular Sciences*, 19(3), p.849, DOI:10.3390/ijms19030849.

Wicke, S., Schneeweiss, G. M., dePamphilis, C. W., Müller, K. F., and Quandt, D. (2011). The evolution of the plastid chromosome in land plants: Gene content, gene order, gene function. *Plant Molecular Biology, 76*(3–5), pp. 273–297, DOI: 10.1007/s11103-011-9762-4.

Xue, M., Guo, T., Ren, M., Wang, Z., Tang, K., Zhang, W. and Wang, M. (2019). Constitutive Expression of Chloroplast Glycerol-3-Phosphate Acyltransferase from Ammopiptanthus Mongolicus Enhances Unsaturation of Chloroplast Lipids and Tolerance to Chilling, Freezing and Oxidative Stress in Transgenic Arabidopsis. *Plant Physiology and Biochemistry*, 143, pp.375–387, DOI:10.1016/j.plaphy.2019.07.019.

Yoo, Y.-H., Nalini Chandran, A.K., Park, J.-C., Gho, Y.-S., Lee, S.-W., An, G. and Jung, K.-H. (2017). OsPhyB-Mediating Novel Regulatory Pathway for Drought Tolerance in Rice Root Identified by a Global RNA-Seq Transcriptome Analysis of Rice Genes in Response to Water Deficiencies. *Frontiers in Plant Science*, 8, DOI:10.3389/fpls.2017.00580.

Zhang, Y., Guo, Y.M., Li, T.J., Chen, C.H., Shen, K.N. and Hsiao, C.D. (2016). The complete chloroplast genome of Gracilariopsis lemaneiformis, an important economic red alga of the family Gracilariaceae. *Mitochondrial DNA Part B, 1(1)*,.2-3.

Zhu, B., Feng, Q., Yu, J., Yu, Y., Zhu, X., Wang, Y., Guo, J., Hu, X., and Cai, M. (2020). Chloroplast genome features of an important medicinal and edible plant: Houttuynia cordata (Saururaceae). *PLoS ONE*, pp.1–16, DOI: 10.1371/journal.pone.0239823.