تحرير الحمض النووي الريبوزي في الميتوكوندريا عبر إنزيم أدينوسين ثلاثي الفوسفات من خلال أنسجة مختلفة

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المستخلص: تحرير الحمض النووي الريبوزي هو عملية تتم ما بعد عملية النسخ حيث يحدث تغير في النيوكليوتيدات للحمض النووي الريبوزي الرسول وقبل وصول هذا الحمض لمرحلة النضج أو الاكتمال. رصدت مواقع مختلفة لنيوكليوتيدات المعدلة في الحمض النووي الريبوزي لجين (*ATP4*) في الميتوكوندريا في كلا من نبات الأرابيدوبسيس ونبات الذرة في دراسات سابقة. يعتبر هذا الجين الذي مهم في تصنيع إنزيم أدينوسين ثلاثي الفوسفات الذي ينتج الطاقة في الميتوكوندريا. في هذه الدراسة، تم تحديد ٤٢ موقع متغير النيوكليوتيدة بسبب تعديل الحمض النووي الريبوزي في نسخ جين *ATP4* في مختلف أجزاء نبات الونكا وذلك عن طريق استخدام التحليل الحاسوبي. كذلك لقد ظهرت تسعة مواقع لتحرير الحمض النووي الريبوزي للجين *ATP4* وهي في مواقع رقم (C59, C71, C89, C215, C248, C251, C395, C407, C500) منها أربعة مواقع جديدة تم اكتشافها في الميتوكوندريا في مختلف الانسجة لهذا النبات بواسطة **high throughput** في الميتوكوندريا لنبات الونكا **sequencing**. وهذه الاختلافات في مواقع تحرير الحمض النووي لهذا الجين تتسبب في تغير الأحماض الامينية الأساسية لغيرها. هذا التغير قد يرفع من نسبة حدوث التنفس الخلوي ومستوى النمو لنبات الونكا.

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C248, C251, C395, C407 and C500) were identified in all *Catharanthus roseus* organs (Table3). A comparative analysis of *ATP4* edits had identification similar positions (C59, C71, C89, C215, C248 and C251) in *Catharansaus roseus* with other species in previous studies. However, we discovered four new RNA editing positions (C71, C395, C407 and C500) from *ATP4* transcripts in *Catharansaus roseus* and that were absent in *Maize* and *Arabidopsis thaliana*. Three exclusive edits of *ATP4* mitochondrial gene transcripts (C395, C407 and C500) were found in all *Catharansaus roseus* organs. *ATP4* edits were appeared most frequently in stem and hairy root organs of *Catharansaus roseus* whereas a low range of *ATP4* RNA editing sites is observed in flower. In seedling, Four RNA editing positions of *ATP4* transcripts (C59, C71, C89 and C215) were absent compared to two positions (C59 and C71) also was not detected in leaf of *Catharansaus roseus* (Table3). The most extensively *ATP4* edits were caused a change from actual amino acids to leucine amino acid expects in position C500 that was changed to methionine amino acid across RNA-seq data of *Catharanthus roseus* tissues. Interestingly, Leucine amino acids are essential in cellular respiration as its important for producing ATP [30](Table4; Figure:1).

V. CONCLUSION:

RNA editing is a post-transcriptional procedure of gene regulation that is occurred before mRNA has been matured by nucleotides conversion. RNA editing status of *ATP4* mitochondrial gene transcript was determined in *Arabidopsis thaliana* and *Maize* in previous study [21]. ATP synthase genes have a significant amount of providing energy for living cells [19]. I was indicated that 42 edits as a total of *ATP4* RNA editing sites in each *Catharanthus roseus* tissues. Nine *ATP4* RNA edits (C59, C71, C89, C215, C248, C251, C395, C407 and C500) were identified, including 4 newly discovered edits, C to T conversion from *ATP4* gene transcript by using a high throughput sequencing in flower, stem, leaf, hairy root,

root and seedling of *Catharanthus roseus*. 597 bp was measured as the length of *ATP4* gene in *Catharanthus roseus*. Variation edits of *ATP4* were characterized and caused a changing on original amino acids to other and could cause an increase in cellular respiration level. Our expected that the increasing RNA editing level will produce good crops. I also suggested that *ATP4* RNA editing will rise the level of cellular respiration and lead to generating more energy (ATP) in both creatures and land plants.

VI. REFERENCES

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Catharanthus roseus and had an accession number of *ATP4* gene with no MK424820 and its accession numbers in each organs, including flower (accession no MK424826), stem (accession no MK424825), leaf (accession no MK424824), hairy root (accession no MK424823), root (accession no MK424822) and seedling (accession no MK424821) in NCBI (<https://www.ncbi.nlm.nih.gov>). NGS data was used to confirm the right sequences depending on CLC genomic workbench program (<http://www.clcbio.com/products/clc-genomics-workbench>) [27].

C. Analysis of RNA editing in *ATP4* transcript and deduced amino acids:

ATP4 RNA editing loci were computationally analysis across *Catharansaus roseus* tissues. Nine editing sites was identified (nucleotideno. C59, C71, C89, C215, C248, C251, C395, C407 and C500) from *ATP4* transcript in *Catharansaus roseus* mitochondria (Table3). Three edits of *ATP4* mitochondrial gene (C395, C407 and C500) were occurred in all *Catharansaus roseus* organs. A high level of edits was detected in stem and hairy root organs of *Catharansaus roseus* whereas a low range of *ATP4* RNA editing sites was observed in flower. Four RNA editing positions (C59, C71, C89 and C215) of mitochondrial *ATP4* were absent in seedling organ as well two positions (C59 and C71) also were not observed in leaf of *Catharansaus roseus* (Table3). Protein alignment of *ATP4* was done and observed in most *ATP4* RNA editing sites and were caused a change in amino acids to leucine amino acid. Only, one edit at position C500 was changed the actual amino acid to methionine amino acid (Table4; Figure:1).

IV. DISCUSSION

ATP4 gene belongs to mitochondrial genes and it is a subunit of complex V family of the electron transport chain in mitochondria [15]. It is an essential gene for encoding subunit 4 of Fo sector in complex V [20]. *ATP4* is a subunit of

ATPase group in all living cells and the major function of this group is generated energy for the cell. RNA editing is a method that is occurring in *ATP4* gene transcript depends on plant need to ATP [28]. This editing causes important benefits to having a good crop with good properties [21].

ATP4 sequence of gene regulation was used to identify the similarity with identification of homologous genes from different plants by specialized BLAST search hits. *Rhazyastricta* and *Catoropis Procera* were illustrated the most closely species that is related to *ATP4* in *Catharanthus roseus* with the 0.0 e- value and 98 percentage of identity. 97 percentage of homologous level was appeared between *ATP4* gene in *Catharanthus roseus* and other variation plants with 0.0 e-value (Table1)[26].

According to Beijing Institute of Genomics that 21,19 and 15 *ATP4* RNA edits were occurred in Ginkgo biloba, *Ophioglossum californicum* and *Nelumbo nucifera*, respectively. There were some similarities on the total number of edits in eight species from 10 to 14 edits. Furthermore, *Cycas taitungensis*, *Spirodela polyrhiza* 7498 and *Welwitschia mirabilis* had only one *ATP4* RNA editing site [29](Table2). In this study, the most extensively edited was occurred with a total of 42 RNA editing sites in *Catharanthus roseus* compared to previous data.

The mitochondrial RNA editing status of *ATP4* transcript was reported and detected in one edit (C59) in *Maize* mitochondria and three edits in *Arabidopsis* (C89, C138 and C250) [21]. Beijing Institute of Genomics was characterized nine positions of RNA editing (C89, C138, C144, C215, C248, C250, C251, C382, C395, C416 and C525) in *Arabidopsis thaliana* [29]. In this study, a computational analysis of *ATP4* edits was done through *Catharansaus roseus* organs with C to T conversion by using CLC genomic workbench program. Nine *ATP4* edits (nucleotideno. C59, C71, C89, C215,

sequences

(<http://www.ebi.ac.uk/Tools/msa/clustalw2/>)[25].

D. RNA Sequence data:

RNA-seq data of *Catharanthus roseus* gathered and downloaded from GeneBank at the National Center for biotechnology information advances science and health (NCBI) as following.

SRR122253 stem

SRR122258 hairy root

SRR342017 leaf

SRR1271858 root

SRR1271859 flower

SRR5133636 seedling

E. Next-Generation Sequencing (NGS)

Whole-RNA-seq and DNA-seq reads of *Catharanthus roseus* were produced by using the Illumina Genome Analyser IIx (GAIIx) based on the manufacturer's instructions (Illumina, San Diego, CA) [24].

F. Sequence filtering and bioinformatics analysis

The sequencing data were acquired using the Illumina python pipeline?? v. 1.3. For the acquired libraries, only high quality reads (quality >20) were conserved. Then, reference assembly using *ATP4* mitochondrion gene of *Rhazya stricta* (accession No. KJ485850) as a reference gene of the obtained short (paired-end) read dataset was performed using assembler CLC Genomics workbench 3.6.5[24].

G. Analysis of RNA editing and deduced amino acids

In the present study, the genomic and cDNA sequences of *ATP4* mitochondrial gene were obtained and analyzed in *Catharanthus roseus* for RNA editing status using multi sequence alignment using CLC genomic work bench 3.6.5(<http://www.clcbio.com/products/clc-genomics-workbench>). Also, protein multi sequence alignment was achieved using the same program.

III. RESULTS

ATP4 RNA editing variation sites were observed and characterized across *Catharanthus roseus* organs by using a genetic program. Variation edits of *ATP4* gene was found and caused an alteration of actual amino acids of mRNA templet of this gene to other.

A. Characterization of *Catharanthus roseus ATP4* gene:

Through this study, DNaseq raw data was used to observe *ATP4* gene in *Catharanthus roseus* (accession no. MK424820). The Whole paired-end short DNA sequence reads of *Catharanthus roseus* was chastized as (71,349,934 paired-end) from using the HiSeq 2000 Illumina platform (Illumina, San Diego, CA). *ATP4* gene of *Catharanthus roseus* was used *ATP4* of *Rhazya stricta* (KJ485850.1) as a reference in CLC genomic workbench. 42 edits of *ATP4* was observed by using High throughput sequencing in *Catharanthus roseus* which was from C to T conversion. *ATP4* gene also was detected in 10 different species as it's in *Catharanthus roseus* by using the BLAST search hits to appear the multisequence alignment (Table1)[26].

B. Characterization of *Catharanthus roseus ATP4* mRNA

RNA sequences raw data was used to characterize cDNA *ATP4* gene through *Catharanthus roseus* organs. (Flower with accession no. SRR1271859, stem with accession no. SRR122253, leaf with accession no. SRR342017, hairy root with accession no. SRR122258, root with accession no. SRR1271858, and seedling with accession no. SRR5133636) were used as reference in CLC genomic workbench. The paired-end short RNA sequence reads of *Catharanthus roseus* tissues was known as (84,355,826 reads in flower, 57,807,858 reads in stem, 123,982,796 reads in leaf, 130,804,028 reads in hairy root, 89,052,194 reads in root and 132,062,664 reads in seedling) from using the HiSeq 2000 Illumina platform (Illumina, San Diego, CA). I submitted *ATP4* gene sequences of

region that has the largest genome compared to the second area which is located in mitochondria [10]. Around 500 edits from mitochondrial genes transcripts have been detected in *Arabidopsis thaliana* and closely 635 edits have been reported in *Angiosperms* [11-13].

Various studies reported that mitochondrial genes separated to five complexes (complex I, II, III, etc) each complex has its own genes [14]. Complex V is ATP synthase complex of cellular respiration, including subunit genes (*ATP1*, *ATP2*, *ATP3*, *ATP4*, etc) [15]. The ATP synthase structure has two subunits, the first one is called F1 catalytic portion and has many subunits including c, d and e. Fo proton-translocator is the second subunits that has a, c, e, f, g and A6L subunits. A peripheral stalk is linkage area between F1 and Fo [16]. The mitochondrial genome of the plant has been encoded by a (*ATP1*), a (*ATP6*), b (*ATP4*), c (*ATP9*), and A6L (*ATP8*) subunits [17, 18]. ATPs genes group is involved on Complex V enzyme which is the last process of oxidative phosphorylation and also has the responsibility of proving energy to the organ and might have a relation of slow or increase the growth rate of the organs [19].

ATP4 gene is one of mitochondrial genes and a subunit of complex V of the electron transport chain in mitochondria [15]. A subunit 4 of Subunit Fo sectora in the ATP synthase was encoded by *ATP4* gene [20]. The deletion of this gene might slow the growth level of plants and the editing sites of this gene could have an important impact on crops with good properties [21].

Catharanthus roseus is a wellspring of synthetic mixes used in growth treatment. The revelation of *Catharanthus roseus* comes about to a therapeutic achievement in the twentieth time [22]. *Catharanthus roseus* compounds are the most extensively used in cancer chemotherapy method such as leukemia, Hodgkin's disease and solid tumors [13], [23]. In this study, a bioinformatics tool was used to identify the variation in RNA editing sites of *ATP4*

gene transcripts over *Catharanthus roseus* tissues and these editing loci cause amino acids conversions. Amino acids changes might increased a cellulare respiration level and the growth rate of *Catharanthus roseus*.

II. METHODOLOGY

A. Plant sample:

Leaf, flower, stem, hairy root, root and seedling of *Catharanthus roseus* were gathered from Jeddah area in Saudi Arabia and were iced in 50 mg liquid state of nitrogen.

B. DNA isolation and sequencing:

3µl RNase A were inserted to the RNA samples tubes to remove the DNA contaminants and incubated at 30 degrees Celsius for a quarter after adding DNase and protease-free to this tube. DNA isolation was done by measuring optical density at 260 nm. DNA was delivered to Beijing Genomics Institute (BGI) to provided us with profound DNA sequences data [24].

C. RNA isolation and validation:

Catharanthus roseus seeds will be put on a dish that has a wet filter paper and then growth under room temperature. After 7 days, Pieces of seedlings were used to segregate DNA and RNA. Genomic DNA will be extractor by DNeasy kit (Qiagen). To isolate RNA, RNeasy Plant Mini Kit (Qiagen, cat. no. 74903) was used to RNA extraction to remove the genomic DNA and to form cDNAs.

The PCR templates were used for RNA editing sites validation were the extracted DNA and acquired cDNAs were be chosen randomly. Edits were specified by RNA-seq read mapping. Particular primers were designed based on the candidate regions. The resulting outcomes were detached by using gel electrophoresis and were sanitized with a Universal DNA Purification Kit (Tiangen, Beijing, China) and were sequenced from both direction. The ClustalW program was used to RNA editing sites validation were validated by aligning cDNAs with identical DNA

RNA Editing in mitochondrial ATP synthase subunit 4 across *Catharanthus roseus* tissues

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Abstract— RNA editing is a post-transcriptional procedure that is occurred before mRNA has been matured by nucleotides changes. Previously, RNA editing status of ATP4 mitochondrial gene transcript was determined in *Arabidopsis thaliana* and Maize. Subunit 4 of ATP synthase genes is essential for complex V production that is indispensable for generated ATP in mitochondria. In this study, in total, computational analysis of 42 RNA editing sites were identified from ATP4 transcripts in all *Catharanthus roseus* organs. Nine edits (C59, C71, C89, C215, C248, C251, C395, C407 and C500) of ATP4 were detected including 4 newly discovered edits, C to T conversion by using a high throughput sequencing in mitochondria transcripts in *Catharanthus roseus*. Differential edits of ATP4 were observed and caused a change of actual amino acids of mRNA templet of this gene to the other. This cause could increase the occurring cellular respiration and the growth level of the *Catharanthus roseus* plants.

Keywords—RNA editing, ATP synthase subunit 4 gene and *Catharanthus roseus* tissues.

I. INTRODUCTION

RNA editing is a process that has an impact organelle transcripts by nucleotides modification on mRNA immature. This modification is typically performed by insertion, deletion and a base substitution of nucleotides within the edited RNA molecule by changing from one nucleotide to the other [1]. The function of RNA editing is generally still poorly understood. Common editing sites are mostly at level of single nucleotide in both plastid and mitochondria in plant [2] [3] [4]. Edits

could produce a new mRNA template that results in having a new protein. This protein might acquire a different character which could influence protein function [5] [6].

RNA editing of gene regulation is existed in human, animals, fungi, bacteria and plant but it is mostly in plants [7]. The most common RNA editing is a range of changes over particular nitrogen-containing base from cytidine to uridine conversion in both plastid and mitochondria blossoming plant [8] [9]. In plant, nucleus is the first