تحورات الحمض النووي الريبي لجينات ndhF, ndhD و ndhC في النبات البلاستيدية

الويكنا

وسيلة بريكان الشمري ١, ٢, هشام الحربي ١، هدى عطالة الحمدان ١، ثناء خالد ١، أحمد رمضان ٢

قسم علوم الأحياء، كلية العلوم، جامعة الملك عبد العزيز، بجدة، السعودية
قسم الأحياء، كلية العلوم، جامعة حائل، حائل، السعودية
قسم الأحياء الجزيئية النباتية، معهد الهندسة الوراثية النباتية للأبحاث، مركز
البحث الزراعي، الجزيرة، مصر

الخلاصة:

التعديل الجيني في البلاستيدات يتضمن إجراء يهدف بعد عملية التسمم يسمى تحرير الحمض النووي الريبي في عملية التحرير النووي الريبي يتم استبدال النوكليوتيدات من سابتسين إلى بورين. هذا التحول يثير انتباه بروتينات كفاءة ودقة أعلى. يشير هذا الإجراء لتسيل النوكليوتيدات على مستوى الحمض النووي الريبي ليختلط عن تسيل الجين المغاير. في هذه الدراسة تم تحديد أمكنة التحويل في الجينات النباتية ndhF و ndhC. وجد التحويل في نباتات W. يسبب إنتزاع النيكلوجين (NAD(P)H). وجد ذلك، تم معالجة الأماكن المعينة في الأنسجة المختلفة (الزهرة، البرقية، الساق، الجذع، الشعارات النجمية، والليال) عن طريق استخدام برنامج CLC genomic workbench ٣.٦.٥.

أطلقت أبحاث سابقة أن تحرير الحمض الريبي يعتمد على نوع النبات ولكن في هذه الدراسة تم توضيح أنه يعتمد أيضاً على نوع الأنسجة في نفس النبات. بناءً على التحليل الحساسي، تم تحديد موقع معين في نبات ndhF و ndhD. لم يتم ملاحظة أي موقع متغير في جميع أنواع النباتات. علاوة على ذلك، تم مقارنة الأحماض الأمينية الناتجة عن هذه التجارب في الأنسجة المختلفة حيث لوحظ أن عامل التحرير زاد من إنتاج الأحماض الأمينية الكارهة للذين حيثماما أن هذه الإجراء قد يكون له تأثير على الأنيمات المحددة. للذين حيثماما أن هذه الإجراء قد يكون له تأثير على الأنيمات المحددة.
VII. References


In ndhF, the comparison among cDNA transcripts and ndhF genome exhibited 2 editing sites in flower (nucleotide number: C1301 and C1802), 3 sites in leaf (nucleotide number: C1301, C1802, C1888), 3 sites in stem (nucleotide number: C290, C1301, C1802), 3 sites in root (nucleotide number: C1301, C1802, C1888) and 4 editing sites (nucleotide number: C290, C1301, C1802, C1888) were revealed in seedling Table 2 (a). As shown in table 2 the great number of edits presented in seedling tissue while the less edits found in flower.

All these editing sites have occurred in protein-coding regions, indicating that RNA editing is more likely to affect the structure and function of deduced protein. As a result, we have demonstrated that the ndhD and ndhF chloroplast transcripts of different tissues of C. roseus undergo to RNA editing which produce more hydrophobic amino acids residues. A previous study reported that RNA editing sites are usually found in positions important for protein formation and can convert amino acids from hydrophilic to hydrophobic form [8]. RNA editing increases the proportion of hydrophobic amino acid codons [22] and this action is important to protein and enzyme function in chloroplast like NAD(P)H dehydrogenase complex [23]. Substitution of Ser to Leu and Pro to Leu change the physicochemical properties of amino acids from hydrophilic to hydrophobic which increases the hydrophobicity of interface residues, one of the important properties of protein-protein interfaces [8]. The 4 amino acid conversions in ndhD were Serine to Leucine, all of which are changing from hydrophilic to hydrophobic residues. The presence of leucine in the amino acids sequencing is essential in protein binding or recognition sites [24]. In ndhF, two substitutions being Serine to Leucine, one Thr to Ile and one Leu to Phe. So, our result showed that four sites in ndhD and two in ndhF were edited to leucine in which all of these amino acids being hydrophobic. Therefore, substituted amino acids increased hydrophobicity in the deduced ndhD and ndhF proteins which enhances its function efficiency.

The three most frequent amino acid transitions are being Ser-Luc, Pro-Luc and Ser-Phe [22]. The deduced amino acid sequences of ndhD and ndhF across different organs of C. roseus after editing are converted to hydrophobic form. It is thought that these amino acids might be important for the functioning of the proteins. The amino acid Leucine is important for protein and formation of enzyme whereas phenylalanine is a common aromatic amino acid and have very hydrophobic side chains [24]. Most of the substituted amino acids due to RNA editing were hydrophobic suggesting that the hydrophobicity of deduced proteins increases their efficiency and then their function.

VI. Conclusion

In conclusion, we identified RNA editing sites in ndhC, ndhD and ndhF genes (NADH-dehydrogenase subunits 3, 4, 5) in different tissues of C. roseus chloroplast genome through a bioinformatics prediction software. A total of five editing positions in ndhD and four positions in ndhF, which vary depending on the tissue type, were predicted. Additionally, most of editing has led to codon modification identifying different amino acids. The most common amino acid transition in this study is being Ser-Luc. Alteration of the encoded amino acids inducing the hydrophobicity might have an influence on protein biological function.
RNA Editing of chloroplast ndhC, ndhD and ndhF genes in Catharanthus roseus tissues

Seedling (nucleotide number: C2, C1298, C1310) Table 1.

The initiation codon ATG was found to be created from ACG by RNA editing at C2 of ndhD gene corresponding to the start codon in other species. Editing at ndhD-213 is silent, proposing that editing may no longer be required for function of protein, probably because of occurrences of compensatory mutations somewhere else in the protein complex [21]. It’s obviously that stem tissues have the highest rate of editing sites with 5 edits whereas flower tissues have the lowest with only 2 edit sites.

Table: (2) RNA editing sites of ndhF gene and encoded amino acids in different tissues of Catharanthus roseus

E: editing
a total of 4 amino acid substitutions were recognized, two being Serine to Leucine, one from Threonine to Isoleucine and one from Leucine to Phenylalanine, in which all being converted to hydrophobic residues.

3.2 Analysis of the deduced amino acids sequences

The effect of RA editing is determined at protein level. So, we compared amino acid sequences among ndhc, ndhD and ndhF genes and cDNA transcripts in different tissues (flower, leaf, stem, root, hairy root and seedling) of C. roseus Table 1, Fig.1.

As mention above, we reported no editing at mRNA sequences of ndhc thus no amino acids change has observed. Editing status in ndhD at nucleotide number 2 has been changed to methionine a start codon which important to initiate the translation process while editing at C213 is silent with no impact on the encoded amino acid. On the other hand, editing at nucleotide number 383, 674, 1298 and 1310 converts a Ser codon to a Leu codon. However, editing at ndhF-290 and 1802 modified Ser to a Leu amino acid whilst at ndhF-1301 changed Thr to Ile and at ndhF-1888 altered Leu to phe Table 2, Fig.2. Among the 10 editing positions in both ndhD and ndhF, the most frequently edited codon was Ser switched to Leu. As a result, the hydrophobicity was increased in result of

<table>
<thead>
<tr>
<th>Tissue</th>
<th>290</th>
<th>1301</th>
<th>1802</th>
<th>1888</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower</td>
<td>No Editing</td>
<td>E Thr to Ile</td>
<td>E Ser to Leu</td>
<td>No Editing</td>
</tr>
<tr>
<td>leaf</td>
<td>No Editing</td>
<td>E Thr to Ile</td>
<td>E Ser to Leu</td>
<td>E Leu to Phe</td>
</tr>
<tr>
<td>Stem</td>
<td>E Ser to Leu</td>
<td>E Thr to Ile</td>
<td>E Ser to Leu</td>
<td>No Editing</td>
</tr>
<tr>
<td>Root</td>
<td>E Ser to Leu</td>
<td>E Thr to Ile</td>
<td>E Ser to Leu</td>
<td>No Editing</td>
</tr>
<tr>
<td>Hairy root</td>
<td>No Editing</td>
<td>E Thr to Ile</td>
<td>E Ser to Leu</td>
<td>E Leu to Phe</td>
</tr>
<tr>
<td>Seedling</td>
<td>E Ser to Leu</td>
<td>E Thr to Ile</td>
<td>E Ser to Leu</td>
<td>E Leu to Phe</td>
</tr>
</tbody>
</table>

V. Discussion

RNA editing is an important process that converts cytidine to uridine in higher plants chloroplasts [19]. Hence, it appears that the vital function of chloroplast RNA editing is to produce codons fundamental for protein function [20]. The chloroplast ndhC, dhD and ndhF genes are membrane subunits encode subunit 3, 4 and 5 of a plastid NAD (P) H dehydrogenase, a multiprotein complex involved in chloro-respiration and photosystem I [18].

We have analyzed RNA editing of ndhc, ndhD and ndhF in C. roseus. Editing in ndhc was not reported in previous studies as well in this investigation. We found no editing event in ndhc and this may indicate that ndhc is available as a vital form, so it doesn’t need to be edited. However, editing observed in ndhD and ndhF, whereas the number of RNA editing sites vary among the different organs. Consequently, previous studies reported that RNA editing is dependent on plant species however, in this study we demonstrated that RNA editing is dependent on tissue type as well. In ndhD, we identified 2 editing sites in flower (nucleotide number: C2 and C213), 4 sites in leaf (nucleotide number: C2, C383, C1298, C1310), 5 sites in stem (nucleotide number: C2, C383, C674, C1298, C1310), 4 sites in root (nucleotide number: C2, C213, C674, C1298) and 3 sites in hairy root and...
The complete plastid genome of Madagascar Periwinkle *Catharanthus roseus* was sequenced in 2013 [15]. The size of *ndhC* is 363 bp, *ndhD* is 1503 bp and *ndhF* is 2229 bp. Genome of *ndhC*, *ndhD* and *ndhF* and cDNA transcripts of flower, leaf, stem, root, hairy root and seedling tissues were downloaded and characterized using RNA-seq raw data. A total of 89,191,351 paired-end short flower RNA sequence reads, 122,063,131 paired-end short leaf RNA sequence reads, 44,283,997 paired-end short stem RNA sequence reads, 85,884,081 paired-end short root RNA sequence reads, 75,338,016 paired-end short hairy root RNA sequence reads and 74,297,633 paired-end short seedling were mapped to the chloroplast *ndhC*, *ndhD* and *ndhF* (accession no. KC561139.1) to examine RNA events. Further, alignment across transcripts of different tissues with target genes were made to characterize the modifications at the amino acid level due to editing.

### 3.1 Prediction of RNA editing in *ndhC*, *ndhD* and *ndhF* transcripts of *C. roseus*

Alignment of cDNA transcripts of different tissues of *C. roseus* (flower, leaf, stem, root, hairy root and seedling) revealed no editing status in *ndhC*. However, for *ndhD* we identified 2, 4, 5, 4, 3, 3 edits in flower, leaf, stem, root, hairy root and seedling, respectively; 2 sites in flower (nucleotide number: C2, C213), 4 sites in leaf (nucleotide number: C2, C383, C1298, C1310), 5 sites in stem (nucleotide number: C2, C383, C674, C1298, C1310), 4 sites in root (nucleotide number: C2, C213, C674, C1298), 3 sites in hairy root (nucleotide number: C2, C1298, C1310) and 3 sites in seedling (nucleotide number: C2, C1298, C1310) Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Editing Sites C to T and amino acid changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Flower</td>
<td>E</td>
</tr>
<tr>
<td>Leaf</td>
<td>E</td>
</tr>
<tr>
<td>Stem</td>
<td>E</td>
</tr>
<tr>
<td>Root</td>
<td>E</td>
</tr>
<tr>
<td>Hairy root</td>
<td>E</td>
</tr>
<tr>
<td>Seedling</td>
<td>E</td>
</tr>
</tbody>
</table>

3.1 Predicting RNA editing in *ndhC*, *ndhD* and *ndhF* transcripts of *C. roseus*

Alignment of cDNA transcripts of different tissues of *C. roseus* (flower, leaf, stem, root, hairy root and seedling) revealed no editing status in *ndhC*. However, for *ndhD* we identified 2, 4, 5, 4, 3, 3 edits in flower, leaf, stem, root, hairy root and seedling, respectively; 2 sites in flower (nucleotide number: C2, C213), 4 sites in leaf (nucleotide number: C2, C383, C1298, C1310), 5 sites in stem (nucleotide number: C2, C383, C674, C1298, C1310), 4 sites in root (nucleotide number: C2, C213, C674, C1298), 3 sites in hairy root (nucleotide number: C2, C1298, C1310) and 3 sites in seedling (nucleotide number: C2, C1298, C1310) Table 1.
Pentatricopeptide repeat (PPR) proteins that are encoded in the nuclear genome play a central role in the post-transcriptional regulation of gene expression [9]. They are essential for the recognition of RNA editing sites and most these proteins are thought to localize in plants' plastid and mitochondria [10]; [11].

Chloroplasts are large organelles in plants responsible for photosynthesis and generate metabolic energy. They contain their own genome called plastome and their own gene expression machinery [12]. These organelles undergo RNA editing converting cytosine to uracil (C-to-U) and less frequently uracil to cytosine (U-to-C) ranging from hundreds editing sites in Hornwort and fern [6, 13] to about 30 to 40 editing sites in angiosperms [14]. Chloroplasts RNA editing has been reported in all land plants studied yet. It was first detected in rpl2 gene in the maize plastid [5]. Many of the chloroplast genes were identified due to completely sequenced of number of plants chloroplasts. [15]. These chloroplast genes encode proteins complexes needed for cellular processes such as photorespiration, photosystem and other necessary organellar functions. NAD (P) H dehydrogenase (NDH) complex is one of these important complexes. It is encoded by 11 genes homologs to the subunits of mitochondrial NADH dehydrogenase (complex I) [16, 17] [18]. The chloroplast ndhC, ndhD and ndhF genes are membrane subunits encode subunit 3, 4 and 5 of a plastid NAD (P) H dehydrogenase, a multiprotein complex involved in chloro-respiration and photosystem I [18].

In this paper, we investigated RNA editing sites in ndhC, ndhD and ndhF transcripts from flower, leaf, stem, root, hairy root and seedling organs of C. roseus chloroplast plastome (accession no. KC561139.1). Further RNA editing sites were compared among the different tissues. Our results detected no editing status in ndhC and differential editing events among the six tissues in ndhD and ndhF. All editing events in both genes ndhD and ndhF have changed the deduced amino acids except one was silent editing in ndhD; didn’t alter the amino acid. In addition, bioinformatics analysis exhibited that RNA editing increases hydrophobicity of resultant amino acids.

II. Materials and Methods

2.1 Selecting Database for ndhB chloroplast gene and RNA-seq

Sequence of ndhC, ndhD and ndhF genes and RNA-Seq of C. roseus plant tissues (flower, leaf, stem, root, hairy root and seedling), were taken and downloaded from GeneBank at the National Center for Biotechnology Information Advances Science and Health (NCBI). Accession number are listed as follows: C.roseus chloroplast ndhC, ndhD and ndhF (KC561139.1); RNA-Seq: SRR122239 flower, SRR122251 leaf, SRR122253 stem, SRR122254 root, SRR122257 hairy root, SRR122243 seedling.

2.2 Bioinformatics Analysis of RNA editing

Sequences of ndhC, ndhD and ndhF genes and cDNA of different C. roseus tissues applied in this paper were analyzed through multi-sequence alignment using CLC genomic workbench 3.6.5, a software for predicting RNA edit sites (http://www.clcbio.com/products/clc-genomics-workbench). The edit events detected in transcripts were compared among 6 different tissues.

2.3 Analysis of the deduced amino acids sequences

A comparison of amino acid sequences was made among cDNA transcripts of different organs (flower, leaf, stem, root, hairy root and seedling) of C. roseus after protein sequences have been deduced. The same software (Multi-sequences alignment using CLC genomic workbench 3.6.5) was employed to identify the effect of editing on the conversion of amino acids across different tissues. Finally, the identified amino acids substitutions were compared across the different organs.

2.4 Accession Numbers

Sequences data from this article have been submitted to GenBank data library under following accession numbers; transcripts of ndhC (flower) MK434978, (leaf) MK434979, (stem) MK43498, (Root) MK434981, (hairy
RNA Editing of chloroplast ndhC, ndhD and ndhF genes in Catharanthus roseus tissues

Wasimah B. Alshammari 1,2, Hesham F. Alharby 1, Huda A Alhamdan 1, Thana K. Khan 1, Ahmed M. Ramadan 1,3

1Biological Sciences Department, Faculty of Science, King Abdul-Aziz University, Jeddah, Saudi Arabia
2Biological Sciences Department, Faculty of Science, Hail University, Hail, Saudi Arabia
3Plant Molecular Biology Department, Agricultural Genetic Engineering Research Institute (AGERI), Agriculture Research Center (ARC), Giza, Egypt

Email: alshamarywasima@yahoo.com

ABSTRACT: Gene expression in plant chloroplast involves an important posttranscriptional process called RNA editing. At transcript level, RNA editing modifies cytidine-to-uridine and less from U-to-C which is an essential event to produce functional proteins. This action alters the nucleotide sequence of RNA transcript to differ from its gene sequence. In this study, we identified RNA editing in ndhC, ndhD and ndhF genes of Catharanthus roseus chloroplast which encode subunits of NAD (p) H dehydrogenase complex. Editing status were compared across cDNA transcripts of six tissues (flower, leaf, stem, root, hairy root and seedling). Through CLC genomic workbench 3.6.5 a bioinformatics prediction software. We reported that RNA editing event depends on the tissue type as it depends on plant species types. A total of 5 C-to-U editing positions were identified in ndhD, 4 positions in ndhF and no editing event in ndhC. Furthermore, resultant amino acid from these edits were alignment among the six different tissues. We found that RNA editing increased hydrophobicity in ndhD and ndhF proteins, indicating that RNA editing might have an impact on the function of target genes.

Keywords: Catharanthus.roseus, RNA editing, chloroplast, ndhC, ndhD, ndhF, Hydrophobicity.

I. INTRODUCTION

Transcripts of mRNA are edited by single base substitutions, deletions or insertions at specific sites to produce functional mRNA through a process called RNA editing. Conversion of cytidine (C) to uridine (U) or (U) to (C) in RNA transcript to substitute the identity of nucleotides between RNA and its gene is an important post-transcription process to plastids RNA sequence of higher plants [1]; [2]). It was first identified in Trypanosoma brucei mitochondrial cytochrome oxidase (cox) subunit II gene [3] However, RNA editing in plant was first reported as a cytidine to uridine substitution in mitochondrial transcripts [4] and then was identified in chloroplasts [5]. RNA editing is a mechanism that converts the amino acid residues in mRNAs or creates translation start or stop codons [6], thus, it is an important process to restore genetic information. Most of mechanism at mRNA transcript level which restore conserved amino acid residues [7]. Additionally, RNA editing leads in most cases to the conversion of hydrophilic amino acid to hydrophobic a substantial characteristic of protein-protein interfaces [8].RNA editing can affect protein functions by enhancing enzymatic activity of protein and controlling protein stabilization [8].