تحويلات الحمض النووي الريبسي لجينات *ndhC* و *ndhD* و *ndhF* البلاستيدية في أنسجة نبات الوينكا

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الخلاصة:

التعبير الجيني في البلاستيدات يتضمن إجراء يحدث بعد عملية النسخ يسمى تحرير الحمض النووي الريبوزي في عملية التحرير النووي الريبوزي يتم استبدال النيكلوتيدات من سايتوسين إلى يورادين وهذا التحول يعتبر أساسياً لإنتاج بروتينات بكفاءة وظيفية أعلى. يغير هذا الإجراء تسلسل النيكلوتيدات على مستوى الحمض النووي الريبوزي ليختلف عن تسلسل الجين المنسوخ. في هذه الدراسة تم تحديد أماكن التعديل في الجينات التالية *ndhD*, *ndhC* و *ndhF* من بلاستيد نبات الوينكا الطبي والتي تقوم بترجمة وحدات تدخل في تكوين وبناء مركب إنزيم الديهيدروجيناز (NAD(P) H). وبعد ذلك، تم مقارنة الأماكن المعدلة في الأنسجة المختلفة (الزهرة، الورقة، الساق، الجذر، الشعيرات الجذرية والباذرات) عن طريق استخدام برنامج CLC genomic workbench 3.6.5. بينت أبحاث سابقة أن تحرير الحمض النووي الريبوزي يعتمد على نوع النبات ولكن في هذه الدراسة تم توضيح أنه يعتمد أيضاً على نوع الأنسجة في نفس النبات. بناءً على التحليل الحاسوبي، تم تحديد مواقع معدلة في *ndhD* و *ndhF* ولم يتم ملاحظته أي موقع متغير في *ndhC* في جميع أجزاء هذا النبات. علاوة على ذلك، تم مقارنة الأحماض الأمينية الناتجة عن هذه التغيرات في الأنسجة المختلفة حيث لوحظ أن عملية التحرير زادت من إنتاج الأحماض الأمينية الكارهة للماء مبينا أن هذه الإجراء قد يكون له تأثير على الجينات المستهدفة.

VII. References

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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: C290, C1301, C1802), 3 sites in hairy 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 grate 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 increase 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 soft wear. 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.

		260				280				300	
ndhF	AATTCGGCTA	TTTGATAGAT	CCACTTACTT	CTATTATGTC	AATATTAATC						300
Translation +1	Glu Phe Gly Tyr	Leu Ile Asp	Pro Leu Thr	Ser Ile Met Ser	Ile Leu Ile						
ndhF RNA- Flower											300
Translation +1	Glu Phe Gly Tyr	Leu Ile Asp	Pro Leu Thr	Ser Ile Met Ser	Ile Leu Ile						
ndhF RNA- Leaf											300
Translation +1	Glu Phe Gly Tyr	Leu Ile Asp	Pro Leu Thr	Ser Ile Met Ser	Ile Leu Ile						
ndhF RNA- Stem											300
Translation +1	Glu Phe Gly Tyr	Leu Ile Asp	Pro Leu Thr	Ser Ile Met Leu	Ile Leu Ile						
ndhF RNA- Root											300
Translation +1	Glu Phe Gly Tyr	Leu Ile Asp	Pro Leu Thr	Ser Ile Met Leu	Ile Leu Ile						
ndhF RNA- Hairly Root											300
Translation +1	Glu Phe Gly Tyr	Leu Ile Asp	Pro Leu Thr	Ser Ile Met Ser	Ile Leu Ile						
ndhF RNA- Seedling											300
Translation +1	Glu Phe Gly Tyr	Leu Ile Asp	Pro Leu Thr	Ser Ile Met Leu	Ile Leu Ile						
		1,320				1,340					
ndhF	CAGCCTGGGG	TACCGCTGGA	TTAACC GCAT	TTTATATGTT	TCGAATCTAT						1350
Translation +1	Thr Ala Trp Gly	Thr Ala Gly	Leu Thr Ala	Phe Tyr Met Phe	Arg Ile Tyr						
ndhF RNA- Flower	T.....										1350
Translation +1	Ile Ala Trp Gly	Thr Ala Gly	Leu Thr Ala	Phe Tyr Met Phe	Arg Ile Tyr						
ndhF RNA- Leaf	T.....										1350
Translation +1	Ile Ala Trp Gly	Thr Ala Gly	Leu Thr Ala	Phe Tyr Met Phe	Arg Ile Tyr						
ndhF RNA- Stem	T.....										1350
Translation +1	Ile Ala Trp Gly	Thr Ala Gly	Leu Thr Ala	Phe Tyr Met Phe	Arg Ile Tyr						
ndhF RNA- Root	T.....										1350
Translation +1	Ile Ala Trp Gly	Thr Ala Gly	Leu Thr Ala	Phe Tyr Met Phe	Arg Ile Tyr						
ndhF RNA- Hairly Root	T.....										1350
Translation +1	Ile Ala Trp Gly	Thr Ala Gly	Leu Thr Ala	Phe Tyr Met Phe	Arg Ile Tyr						
ndhF RNA- Seedling	T.....										1350
Translation +1	Ile Ala Trp Gly	Thr Ala Gly	Leu Thr Ala	Phe Tyr Met Phe	Arg Ile Tyr						
		1,820				1,840					
ndhF	TCAAAAGATG	CCATTTTTTC	AGTCAGTATA	GCTTATTTAG	GAATATTGAT						1850
Translation +1	Ser Lys Asp	Ala Ile Phe Ser	Val Ser Ile	Ala Tyr Leu	Gly Ile Leu Ile						
ndhF RNA- Flower	.T.....										1850
Translation +1	Leu Lys Asp	Ala Ile Phe Ser	Val Ser Ile	Ala Tyr Leu	Gly Ile Leu Ile						
ndhF RNA- Leaf	.T.....										1850
Translation +1	Leu Lys Asp	Ala Ile Phe Ser	Val Ser Ile	Ala Tyr Leu	Gly Ile Leu Ile						
ndhF RNA- Stem	.T.....										1850
Translation +1	Leu Lys Asp	Ala Ile Phe Ser	Val Ser Ile	Ala Tyr Leu	Gly Ile Leu Ile						
ndhF RNA- Root	.T.....										1850
Translation +1	Leu Lys Asp	Ala Ile Phe Ser	Val Ser Ile	Ala Tyr Leu	Gly Ile Leu Ile						
ndhF RNA- Hairly Root	.T.....										1850
Translation +1	Leu Lys Asp	Ala Ile Phe Ser	Val Ser Ile	Ala Tyr Leu	Gly Ile Leu Ile						
ndhF RNA- Seedling	.T.....										1850
Translation +1	Leu Lys Asp	Ala Ile Phe Ser	Val Ser Ile	Ala Tyr Leu	Gly Ile Leu Ile						
		1,860				1,880				1,900	
ndhF	AGCGTCCTTT	TTATATAAAG	CTCCTTATTC	CTATTTACTC	AATTTTTATT						1900
Translation +1	Ala Ser Phe	Leu Tyr Lys	Ala Pro Tyr Ser	Tyr Leu Leu	Asn Phe Tyr						
ndhF RNA- Flower											1900
Translation +1	Ala Ser Phe	Leu Tyr Lys	Ala Pro Tyr Ser	Tyr Leu Leu	Asn Phe Tyr						
ndhF RNA- Leaf											1900
Translation +1	Ala Ser Phe	Leu Tyr Lys	Ala Pro Tyr Ser	Tyr Leu Phe	Asn Phe Tyr						
ndhF RNA- Stem											1900
Translation +1	Ala Ser Phe	Leu Tyr Lys	Ala Pro Tyr Ser	Tyr Leu Leu	Asn Phe Tyr						
ndhF RNA- Root											1900
Translation +1	Ala Ser Phe	Leu Tyr Lys	Ala Pro Tyr Ser	Tyr Leu Leu	Asn Phe Tyr						
ndhF RNA- Hairly Root											1900
Translation +1	Ala Ser Phe	Leu Tyr Lys	Ala Pro Tyr Ser	Tyr Leu Phe	Asn Phe Tyr						
ndhF RNA- Seedling											1900
Translation +1	Ala Ser Phe	Leu Tyr Lys	Ala Pro Tyr Ser	Tyr Leu Phe	Asn Phe Tyr						

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

Translation +1	Trp	Lys	Phe	Phe	Pro	Trp	Leu	Trp	Ile	Ile	Val	Val	Leu	Pro	Ile	Phe	Ala	
ndhD RNA- Flower	T																	
Translation +1	Met	Lys	Phe	Phe	Pro	Trp	Leu	Thr	Ile	Ile	Val	Val	Leu	Pro	Ile	Phe	Ala	
ndhD RNA- Leaf	T																	
Translation +1	Met	Lys	Phe	Phe	Pro	Trp	Leu	Thr	Ile	Ile	Val	Val	Leu	Pro	Ile	Phe	Ala	
ndhD RNA- Stem	T																	
Translation +1	Met	Lys	Phe	Phe	Pro	Trp	Leu	Thr	Ile	Ile	Val	Val	Leu	Pro	Ile	Phe	Ala	
ndhD RNA- Root	T																	
Translation +1	Met	Lys	Phe	Phe	Pro	Trp	Leu	Thr	Ile	Ile	Val	Val	Leu	Pro	Ile	Phe	Ala	
ndhD RNA- Hairy Root	T																	
Translation +1	Met	Lys	Phe	Phe	Pro	Trp	Leu	Thr	Ile	Ile	Val	Val	Leu	Pro	Ile	Phe	Ala	
ndhD RNA- Seedling	T																	
Translation +1	Met	Lys	Phe	Phe	Pro	Trp	Leu	Thr	Ile	Ile	Val	Val	Leu	Pro	Ile	Phe	Ala	
ndhD	TTATAAATGG							ATCCGTTTT			TTGATTTCCA			CTGGAGATTA			GGAATAGATG	
Translation +1	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
ndhD RNA- Flower	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
Translation +1	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
ndhD RNA- Leaf	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
Translation +1	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
ndhD RNA- Stem	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
Translation +1	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
ndhD RNA- Root	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
Translation +1	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
ndhD RNA- Hairy Root	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
Translation +1	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
ndhD RNA- Seedling	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
Translation +1	Tyr Lys Trp							Ile Arg Phe			Phe Asp Phe His			Trp Arg Leu			Gly Ile Asp	
ndhD	GATGTTAGCA							ATGTACAGTG			GTCAAATAGG			ATCATTITCT			TCTCGAGACC	
Translation +1	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
ndhD RNA- Flower	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
Translation +1	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
ndhD RNA- Leaf	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
Translation +1	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
ndhD RNA- Stem	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
Translation +1	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
ndhD RNA- Root	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
Translation +1	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
ndhD RNA- Hairy Root	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
Translation +1	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
ndhD RNA- Seedling	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
Translation +1	Met Leu Ala							Met Tyr Ser			Gly Gln Ile Gly			Ser Phe Ser			Ser Arg Asp	
ndhD	TTTTATTGCT							TTTGCTGTCA			AATCGCCGAT			TATACCCCTA			CATACATGGT	
Translation +1	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
ndhD RNA- Flower	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
Translation +1	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
ndhD RNA- Leaf	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
Translation +1	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
ndhD RNA- Stem	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
Translation +1	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
ndhD RNA- Root	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
Translation +1	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
ndhD RNA- Hairy Root	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
Translation +1	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
ndhD RNA- Seedling	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
Translation +1	Phe Ile Ala							Phe Ala Val			Lys Ser Pro Ile			Ile Pro Leu			His Thr Trp	
ndhD	AATTACTTTT							GTAATGGCAA			TTGGGATGAT			ATTAACCTCT			ATTATTATCAT	
Translation +1	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
ndhD RNA- Flower	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
Translation +1	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
ndhD RNA- Leaf	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
Translation +1	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
ndhD RNA- Stem	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
Translation +1	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
ndhD RNA- Root	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
Translation +1	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
ndhD RNA- Hairy Root	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
Translation +1	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
ndhD RNA- Seedling	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
Translation +1	Ile Thr Phe							Val Met Ala			Ile Gly Met Ile			Leu Thr Pro			Ile Tyr Ser	
ndhD	TATCTATGTC							ACGCCAGATG			TTCTATGGAT			ATAAGCTTTT			TAACGTCCCA	
Translation +1	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
ndhD RNA- Flower	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
Translation +1	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
ndhD RNA- Leaf	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
Translation +1	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
ndhD RNA- Stem	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
Translation +1	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
ndhD RNA- Root	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
Translation +1	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
ndhD RNA- Hairy Root	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
Translation +1	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
ndhD RNA- Seedling	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	
Translation +1	Leu Ser Met Ser							Arg Gln Met			Phe Tyr Gly			Tyr Lys Leu Phe			Asn Val Pro	

hydrophobicity was increased in result of

Table: (1) RNA editing sites of *ndhD* gene and encoded amino acids in different tissues of *Catharanthus roseus*
E: editing

Tissue	Editing Sites			
	290	1301	1802	1888
Flower	No Editing	E Thr to Ile	E Ser to Leu	No Editing
leaf	No Editing	E Thr to Ile	E Ser to Leu	E Leu to Phe
Stem	E Ser to Leu	E Thr to Ile	E Ser to Leu	No Editing
Root	E Ser to Leu	E Thr to Ile	E Ser to Leu	No Editing
Hairy root	No Editing	E Thr to Ile	E Ser to Leu	E Leu to Phe
Seedling	E Ser to Leu	E Thr to Ile	E Ser to Leu	E Leu to Phe

a total of 4 amino acid substitutions were recognized, two being Serine to Leucine, one Threonine to Isoleucine and one 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

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 and5 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

root) MK434982, (seedling) MK434983. Transcripts of *ndhD* under accession numbers; MK434972 (flower), MK434973 (leaf), MK434974 (stem), MK434975 (Root), MK434976 (hairy root), MK434977 (seedling). Accession numbers of *ndhF* transcripts; (flower) MK434966, (leaf)

3.1 Predictio 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

Tissue	Editing Sites C to T and amino acid changes					
	2	213	383	674	1298	1310
Flower	E (Thr to Met)	E No amino acid change	No Editing	No Editing	No Editing	No Editing
leaf	E (Thr to Met)	No Editing	E Ser to Leu	No Editing	E Ser to Leu	E Ser to Leu
Stem	E (Thr to Met)	No Editing	E Ser to Leu	E Ser to Leu	E Ser to Leu	E Ser to Leu
Root	E (Thr to Met)	E No amino acid change	No Editing	E Ser to Leu	E Ser to Leu	No Editing
Hairy root	E (Thr to Met)	No Editing	No Editing	No Editing	E Ser to Leu	E Ser to Leu
Seedling	E (Thr to Met)	No Editing	No Editing	No Editing	E Ser to Leu	E Ser to Leu

MK434967, (stem) MK434968, (Root) MK434969, (hairy root) MK434970, and (seedling) MK434971.

III. Result

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 74,297,533 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.

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.

silent editing (*ndhD*-213) with no amino acid change (Ile to Ile) was identified as well. Four editing sites (*ndhD*-383, -674, -1298, -1310) caused amino acid conversion, all being Serine to Leucine, the most common amino acid substitution. Additionally, a comparison made among *ndhF* and cDNA sequences showed 2, 3, 3, 3, 3 and 4 edits in flower, leaf, stem, root, hairy root and seedling, respectively; 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: C290, C1301, C1802), 3 sites in hairy root (nucleotide number: C1301, C1802, C1888) and 4 editing sites in seedling (nucleotide number: C290, C1301, C1802, C1888) Table 2. Due to editing in *ndhF*

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*, *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].

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

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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].