Journal of Animal and Veterinary Advances 12 (15): 1276-1280, 2013
ISSN: 1680-5593

Molecular Characterization and Expression Pattern of a Novel Peroxisomal Nicotinamide Adenine Dinucleotide Carrier Gene

[1]Nian Fuzhao, [2]Zhang Yonghui, [2]Luo Dingqi[2], Xia Jianhua, [2]Jiang Sheng and [1]Zhao Leifeng
[1]College of Tobacco Science, Yunnan Agricultural University, 650201 Kunming, China
[2]Technological Center of Tobacco Company of Sichuang Province, 646000 Luzhou, China

Abstract: Nicotinamide adenine dinucleotide have significant health benefits for animals and humans. Peroxisomal nicotinamide adenine dinucleotide carrier gene functions in mediating the nicotinamide adenine dinucleotide import into peroxisomes. The complete coding sequence of tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene was amplified by RT-PCR. The open reading frame of tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene was 1017 bp which encodes a protein of 338 amino acids. Sequence analysis revealed that the peroxisomal nicotinamide adenine dinucleotide carrier of tobacco shares high homology with the peroxisomal nicotinamide adenine dinucleotide carrier of *Lycopersicon esculentum* (88%), *Fragaria vesca* subsp. vesca (74%), thale cress (73%), chickpea (73%) and foxtail millet (67%). Phylogenetic tree analysis revealed that the tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene has a closer genetic relationship with that of *Lycopersicon esculentum*. Prediction of transmembrane helices showed that tobacco peroxisomal nicotinamide adenine dinucleotide carrier might be a transmembrane protein. Expression profile was studied and the results indicated that tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene was highly expressed in stem, leaf and flower. These results established the primary foundation of using tobacco nicotinamide adenine dinucleotide as drug for animals and humans in the future.

Key words: Tobacco, gene, peroxisomal nicotinamide adenine dinucleotide carrier, expression pattern, millet

INTRODUCTION

Recent evidences indicated that the depletion of the coenzyme nicotinamide adenine dinucleotide results in axonal degeneration (Ding *et al*., 2013; Kaneko *et al*., 2006; Sasaki *et al*., 2006). Axonal degeneration is a common pathological feature of a variety of neuropathological disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease and diabetic neuropathies (Ding *et al*., 2013; Fischer *et al*., 2004; Raff *et al*., 2002; Stokin *et al*., 2005). Moreover, nicotinamide adenine dinucleotide supplementation suppresses the development of axonal degeneration in traumatic injury, ischemia damage, autoimmune encephalomyelitis, p53-induced neuron apoptosis and radiation-induced immunosuppression (Ding *et al*., 2013; Luo *et al*., 2001; Klaidman *et al*., 2003; Sadanaga-Akiyoshi *et al*., 2003). The reduction of axonal degeneration by nicotinamide adenine dinucleotide is presumably due to its propensity to reduce oxidative stress or oxidative damage in the neurons (Ding *et al*., 2013; Zhang and Lidup, 1996; Kawai *et al*., 2006; Hipkiss, 2010). Addition of exogenous nicotinamide adenine dinucleotide can prevent mefloquine-induced neuroaxonal and hair cell degeneration through reduction of caspase-3-mediated apoptosis in cochlear organotypic cultures (Ding *et al*., 2013).

Based on the described above, it can be seen that nicotinamide adenine dinucleotide is an important drug which has significant health benefits for animals and humans. Nicotinamide adenine dinucleotide is synthesized outside the peroxisome and must be imported across the permeability barrier of the inner peroxisome membrane. Peroxisomal nicotinamide adenine dinucleotide carrier has been characterized to be responsible for this transport function. Peroxisomal nicotinamide adenine dinucleotide carrier mediates the nicotinamide adenine dinucleotide import into peroxisomes. It favors the nicotinamide adenine dinucleotide (in)/AMP (out) antiport exchange and is required for peroxisomes proliferation (Mano *et al*., 2011; Agrimi *et al*., 2012; Bernhardt *et al*., 2012).

Peroxisomal nicotinamide adenine dinucleotide carrier gene has been identified from many plants. Until today, the tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene has not been reported yet. In present experiment, researchers will isolate the complete mRNA sequences of this tobacco gene, subsequently perform some necessary sequence analysis and tissue expression analysis for this gene. These will establish the primary foundation of using tobacco nicotinamide adenine dinucleotide as drug for animals and humans in the future.

Corresponding Author: Zhao Leifeng, College of Tobacco Science, Yunnan Agricultural University, 650201 Kunming, China

Table 1: PCR primers for tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene isolation

Genes	Primer sequence	Ta (°C)	Length (bp)
Peroxisomal nicotinamide adenine dinucleotide carrier	Forward : 5'-ATGTCGGACGCTTTGATC-3'	56	1017
	Reverse: 5'-CTAATGAGGCTTTAATCTGA-3'		

Table 2: qRT-PCR primers for tobacco peroxisomal nicotinamide adenine dinucleotide carrier, actin genes and annealing temperature

Genes	Primer sequence	Ta (°C)	Length (bp)
Peroxisomal nicotinamide adenine dinucleotide carrier	Forward: 5'-GCGTCGCAGGGTGTTTAT-3'	58	285
	Reverse: 5'-CCCATAGGGAGGAGGTTC-3'		
Actin	Forward :5'-CCATTCTTCGTTTGGACCTT-3'	56	257
	Reverse: 5'-TTCTGGGCAACGGAACCT-3'		

MATERIALS AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis: Tobacco plants (Chinese local variety Yunyan 85) were grown in a naturally lit glasshouse with normal irrigation and fertilization. The tissues including leave, stem, root, flower were harvested and immediately frozen in liquid nitrogen and stored at -80°C. Total RNA extraction and first-strand cDNA synthesis for these tissue samples were performed as the methods describe by Liu (2009).

Isolation of the coding sequence: RT-PCR was performed to amplify complete coding sequences of tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene using the cDNA obtained from the pooled tissues above. The 20 μL reaction system was: 2.0 μL cDNA, 2.0 μL 2 mM mixed dNTPs, 2.0 μL 10×Taq DNA polymerase buffer, 1.2 μL 25 mM $MgCl_2$, 1.0 μL 10 mM forward primer, 1.0 μL 10 mM reverse primer, 2.0 units of Taq DNA polymerase (1U/1 μL) and 9.8 μL sterile water. The primers for tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene isolation were designed based on the tobacco EST sequences (GeneBank numbers FG625827 and FG157895) which are highly homologues with the coding sequence of peroxisomal nicotinamide adenine dinucleotide carrier gene of *Lycopersicon esculentum* (Table 1). The PCR program initially started with a 94°C denaturation for 4 min, followed by 35 cycles of 94°C L min^{-1}, 56°C (Table 1)/1 min, 72°C L min^{-1} then 72°C extension for 10 min, finally 4°C to terminate the reaction.

Quantitative Real Time PCR (qRT-PCR) for tissue expression profile analysis: qRT-PCR for evaluating the level of mRNA for peroxisomal nicotinamide adenine dinucleotide carrier gene was performed by the ABI Prism 7300 Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA). The 25 μL reaction volume of PCR reaction contained 1 μL SYBR Green real-time PCR Master Mix, 100 ng cDNA template and 200 nM each primer. Conditions for real-time PCR were: an initial denaturation at 95°C for 3 min, 40 cycles of 95°C for 15 sec, optimal annealing temperature for each specific primer for 15 sec (Table 2), 72°C for 20 sec. The gene relative expression levels were quantified relative to the expression of the reference gene, actin (GenBank Accession No. GQ339768), by employing the $2^{-\Delta\Delta Ct}$ Value Model (Livak and Schmittgen, 2001).

Sequence analysis: The gene prediction of cDNA sequence was performed by GenScan Software (http://genes.mit.edu/GENSCAN.html). The theoretical isoelectric point (pI) and Molecular weight (Mw) of the deduced protein was computed using the Compute pI/Mw tool. The protein analysis were performed using the BLAST tool at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and the Clustalw Software (http://www.ebi.ac.uk/clustalw). The prediction of transmembrane helices in protein was carried out by the TMHMM Server V. 2.0 (http://www.cbs.dtu. dk/services/TMHMM/).

RESULTS AND DISCUSSION

Isolation result for tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene: For tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene, through RT-PCR with pooled tissue cDNAs, the resulting PCR products were 1017 bp (Fig. 1).

Sequence analysis: The cDNA nucleotide sequence analysis using the BLAST Software at NCBI server revealed that this gene was not homologous to any of the known tobacco gene and it was then deposited into the Genbank database (Accession No.: KF856281).

The sequence prediction showed that the 1017 bp cDNA sequence represents one single gene which encodes 338 a mino acids (Fig. 2). The theoretical isoelectric point (pI) and Molecular weight (Mw) of the deduced proteins of this tobacco gene were also computed using the Compute pI/Mw tool. The pI of tobacco peroxisomal nicotinamide adenine dinucleotide carrier is 9.75. The molecular weight of this putative

protein is 37051.98. Further BLAST analysis of these proteins revealed that tobacco peroxisomal nicotinamide adenine dinucleotide carrier has high homology with the peroxisomal nicotinamide adenine dinucleotide carrier of *Lycopersicon esculentum* (Accession No.: XP_004239938, 88%), *Fragaria vesca* subsp. vesca (Accession No.:XP_004307616, 74%), thale cress (Accession No.: NP_181526, 73%), chickpea (Accession No.: XP_004490955, 73%) and foxtail millet (Accession No.: XP_004984687, 67%) (Fig. 3).

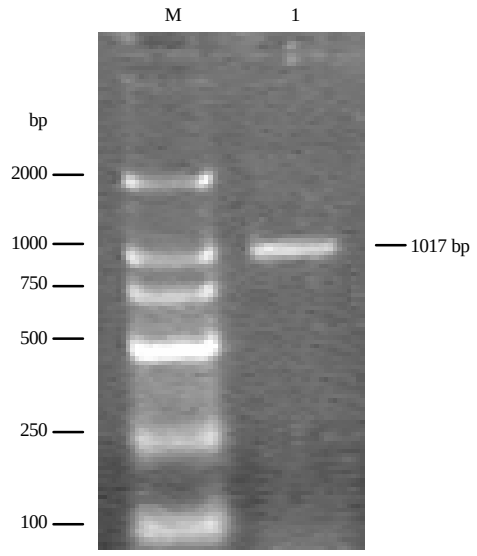

Fig. 1: PCR result for tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene. M: DL2000 DNA markers; 1: PCR product for tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene

Based on the results of the alignment of different peroxisomal nicotinamide adenine dinucleotide carrier proteins, a phylogenetic tree was constructed using the ClustalW Software as shown in Fig. 4. The phylogenetic analysis revealed that the tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene has a closer genetic relationship with that of *Lycopersicon esculentum*.

The prediction of transmembrane helices in protein using the TMHMM Server V. 2.0 showed that tobacco eroxisomal nicotinamide adenine dinucleotide carrier protein might be a transmembrane protein (Fig. 5).

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene was highly expressed in stem, leaf and flower but hardly expressed in root (Fig. 6).

Comparative genomics research has revealed that virtually all (99%) of the protein-coding genes in humans align with homologs in mouse and over 80% are clear 1:1 orthologs for human and mouse both belong to mammalian (Hardison, 2003; Liu, 2009). This extensive conservation in protein-coding regions implied that this conservation of protein-coding sequences may be expected in tobacco and other plants of Solanaceae. From the sequence analysis of peroxisomal nicotinamide adenine dinucleotide carrier genes, it can be seen that the coding sequences of peroxisomal nicotinamide adenine dinucleotide carrier genes were highly conserved in two solanaceae plants-tobacco and *Lycopersicon esculentum*, but not conserved in other plants.

The phylogenetic tree analysis revealed that the tobacco peroxisomal nicotinamide adenine dinucleotide

```
ATGTCGGACGCTTTGATCAGTGGCCTCGCCGGTGCCGGCGGCGGCATCATCGCTCAGCTCATCACCTATCCTCTACAAACAGTAAATACT
 M  S  D  A  L  I  S  G  L  A  G  A  G  G  G  I  I  A  Q  L  I  T  Y  P  L  Q  T  V  N  T
CGGCAGCAAACGGATAGAAATTCTAAGAAAATGAAGCAAACGCTTGGAAGTATCGAACAAATTTGCCAGGTTGTAAAGCAAGAAGGATGG
 R  Q  Q  T  D  R  N  S  K  K  M  K  Q  T  L  G  S  I  E  Q  I  C  Q  V  V  K  Q  E  G  W
GATGGGCTATATGGTGGCTTAACTCCGTCGCTAATTGGTACCGCTGCGTCGCAGGGTGTTTATTATTATTTCTACCAAATATTCAGGAAT
 D  G  L  Y  G  G  L  T  P  S  L  I  G  T  A  A  S  Q  G  V  Y  Y  Y  F  Y  Q  I  F  R  N
AGAGCAGAAACTGATGCCCTTGAACGTAAGAAAAAAGGAATTGGTGATGGATCAGTGGGAATGTTCTCTTCATTACTGGTGGCTGCTTTA
 R  A  E  T  D  A  L  E  R  K  K  K  G  I  G  D  G  S  V  G  M  F  S  S  L  L  V  A  A  L
TCTGGGTGTGTTAACGTGCTGCTGACCAATCCCATATGGGTAGTTGTTACTCGCATGCAGACTCATACAAGAAAGATAAAGATAAACCAC
 S  G  C  V  N  V  L  L  T  N  P  I  W  V  V  V  T  R  M  Q  T  H  T  R  K  D  K  D  N  H
ACCAAACCTGTAACATCACAGGATGCAATATCTGCTGTGGTTGAACCTCCTCCCTATGGGACAAGCTACACTATTCAAGAAGTCTACGGT
 T  K  P  V  T  S  Q  D  A  I  S  A  V  V  E  P  P  P  Y  G  T  S  Y  T  I  Q  E  V  Y  G
GAAGCTGGAGTTTGGGGTTTCTGGAATGGTGTTTTTCCAACATTATTTATGGTAAGCAATCCTTCCATACAATTTATGCTATATGAAACC
 E  A  G  V  W  G  F  W  N  G  V  F  P  T  L  F  M  V  S  N  P  S  I  Q  F  M  L  Y  E  T
TTGCTGAAGAAGATCAGGAAACGACGTGCCTCTAGCAACAAAGGTGCCAATGATGTCACTGCTTTGGAGATATTTCTACTCGGAGCTGTA
 L  L  K  K  I  R  K  R  R  A  S  S  N  K  G  A  N  D  V  T  A  L  E  I  F  L  L  G  A  V
GCAAAACTTGGAGCTACGGTTGTAACGTATCCTCTTCTGGTTATCAAGTCACGACTTCAGGCAAAGCAGGATGTTGGTGGGGATAAAAGA
 A  K  L  G  A  T  V  V  T  Y  P  L  L  V  I  K  S  R  L  Q  A  K  Q  D  V  G  G  D  K  R
CATCACTATAAAGGCACACTTGACGCTATAGTGAAGATTTTACGCTATGAAGGCTTTCATGGATTCTGCAAGGGGATGAGCACAAAAATT
 H  H  Y  K  G  T  L  D  A  I  V  K  I  L  R  Y  E  G  F  H  G  F  C  K  G  M  S  T  K  I
GTACAAAGTGTTCTTGCTGCTGCTGTTCTATTCATGGTCAAGGAAGAACTTGTCAGGGGTGCTAGATGGTTATTAACAGGTCGTTCTGCT
 V  Q  S  V  L  A  A  A  V  L  F  M  V  K  E  E  L  V  R  G  A  R  W  L  L  T  G  R  S  A
ACTTCTGTCAGATTAAAGCCTCATTAG
 T  S  V  R  L  K  P  H  *
```

Fig. 2: The complete coding sequence of tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene and its encoding amino acids. '*' indicates the stop codon

```
Common tobacco                  MSDALISGLAGAGGGIIAQLITYPLQTVNTRQQTDRNSKKMKQTLGSIEQ
Lycopersicon esculentum         MSDALICGIAGAGAGIIAQLITYPLDTVNTRQRTDRNSKKMKKTLGTIEH
Thale cress                     MSDALINGLAGAGGGIIAQLLTYPLQTVNTRQQTERDLKREKRKLGTIEH
Fragaria vesca subsp. Vesca     MSDAVINGLAGAGGGIIAQLITYPLQTVNTRQQTERDLKKARRKLGTVGQ
Chickpea                        MSDALINGLAGAGGGIIAQLITYPLQTVNTRQQTDRDPKKDKKNLGAFQQ
Foxtail millet                  MSDALINGLAGAGGGIIAQLLTFPLQTVNARQQTERDPSKPAFKDGAARQ
                                ****:* *:****.******:*:**:***:**:*:*: .:   . *:  :

Common tobacco                  ICQVVKQEGWDGLYGGLTPSLIGTAASQGVYYYFYQIFRNRAETDALERK
Lycopersicon esculentum         MCQVVKQEGWDRLYGGLAPSLVGTAASQGVYYYFYQILRNRAETDALVRK
Thale cress                     MCQVVKQEGWERLYGGLAPSLAGTAASQGVYYYFYQVFRNRAEATALARK
Fragaria vesca subsp. Vesca     MCQVIRNEGWERLYGGLAPSLVGTAASQGVYYYFYQIFRNKAQVAALERS
Chickpea                        MCQVVKQEGWERLYGGLTPSLVGTATSQGVYYYFYQIFRNRAEEAALEKK
Foxtail millet                  LYLVVKNEGWERLYGGLMPSLVGTAASQGVYYYFYQIFRNRAEAKALERS
                                :  *:::***: ***** *** ***:**********::**:*:  ** :.

Common tobacco                  KKGIGDGSVGMFSSLLVAALSGCVNVLLTNPIWVVVTRMQTHTRKDKDNH
Lycopersicon esculentum         KNGIGDGSVGMFSSLLVAALSGCVNVLLTNPIWVVVTRMQTHSRKDKDNH
Thale cress                     KKGLGDGSVGMFASLLVAAFAGSVNVLMTNPIWVIVTRMQTHRKMTKDQT
Fragaria vesca subsp. Vesca     KMGIGDGSVGMLSSLVVAALSGCVNVLLTNPIWVVVTRMQTHRKISKKSH
Chickpea                        MLGHGDGSVGMISSLIVAALSGCVNVLLTNPIWLVVTRMQTHRKESKRSL
Foxtail millet                  RRGLGDGSVGMLQSLTVAALSGCVNVLLTNPIWVAVTRMQTHRKANIQQS
                                  * *******: ** ***::*.****:*****: ******* :    .

Common tobacco                  TKPVTSQD---AISAVVEPPPYGTSYTIQEVYGEAGVWGFWNGVFPTLFM
Lycopersicon esculentum         PKHEASQD---EISVVVEPPPYGTSHTIQEVYGEDGISGFWNGVFPTLFM
Thale cress                     AAPESPSSNA-EALVAVEPRPYGTFNTIREVYDEAGITGFWKGVIPTLIM
Fragaria vesca subsp. Vesca     PDQMLSTDPEEAIVSAAEPPPFGTTHAVQEVFGEGGFFGFWRGVFPTLIM
Chickpea                        SDWRLPDTTEQKMLSTFEPLAYGTSNVIQEVYNESGVLGFWKGVLPTLVM
Foxtail millet                  PQDLICANDKALEASTTENTPYKTIDVFQELYKEAGVLGFWKGVIPSLIM
                                .           . *  .: *  ..:*:: * *. ***.**:*:*.*

Common tobacco                  VSNPSIQFMLYETLLKKIRKRRASSNKGANDVTALEIFLLGAVAKLGATV
Lycopersicon esculentum         VSNPSIQFMLYETLLKKIRKRRASSNKGANDVTALEIFLLGAVAKLGATV
Thale cress                     VSNPSMQFMLYETMLTKLKKKRALK--GSNNVTALETFLLGAVAKLGATV
Fragaria vesca subsp. Vesca     VSNPSMQFMLYETMLKKLKKRRALSNKTNNGVTAVEIFLLGALAKLGATV
Chickpea                        VSNPSIQFMLYETLLAKLKKEHASN-----GVSALEVFLLGALAKLGATV
Foxtail millet                  VSNPAIQFMLYETLLKRIKKRRASNLKGADGLTALEIFLLGAVAKLGATV
                                ****::*******:* :::*.:* .     .::*:* *****:*******

Common tobacco                  VTYPLLVIKSRLQAKQDVGGDKRHHYKGTLDAIVKILRYEGFHGFCKGMS
Lycopersicon esculentum         VTYPLLVIKSRLQAKQDVGADKRHQYKGTLDALMKIIRYEGFHGFYKGMG
Thale cress                     TTYPLLVVKSRLQAKQVTTGDKRQQYKGTLDAILKMIRYEGLYGFYKGMS
Fragaria vesca subsp. Vesca     VTYPLLVVKSRLQAKQVTTGDKRHHYKGTLDAILKMIRYEGLHGFYKGMS
Chickpea                        VTYPLLVVKARLQARQVKNGDKRHNYEGTWDAIIKMIRYEGLNGFYKGMG
Foxtail millet                  LTYPLLVVKARLQVKQRIDGDKRHHYKGTFDAITKLVQYEGLRGMYKGMG
                                 ******:*:***.:*   .***::*:** **: *:::***: *: ***.

Common tobacco                  TKIVQSVLAAAVLFMVKEELVRGARWLLTGRSATSVRLKPH--
Lycopersicon esculentum         TKIVQSVLAAAILFMVKEELVRCARWLLTARDVANSIS-----
Thale cress                     TKIVQSVLAAAVLFMIKEELVKGAKLLLSNATSS---------
Fragaria vesca subsp. Vesca     TKIVQSVLAAAVLFMVKEELVKGVRFMLTNKVKSKPF------
Chickpea                        TKIVQSVLAAAVLFMVKEELVKQTRFLLTKPKPL---------
Foxtail millet                  TKIVQSVFASALLFMIKEELVKGARLLVTGNTSLVKKLPSKPS
                                ********:*:*:***:*****: .: :::
```

Fig. 3: The alignment of the proteins encoded by tobacco and other peroxisomal nicotinamide adenine dinucleotide carrier genes

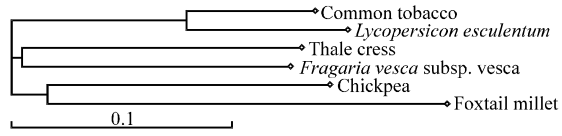

Fig. 4: The phylogenetic tree for five kinds of peroxisomal nicotinamide adenine dinucleotide carrier genes

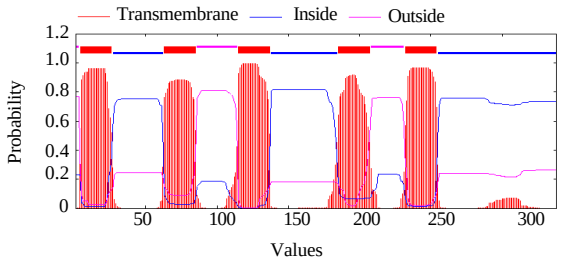

Fig. 5: The transmembrane protein prediction of tobacco eroxisomal nicotinamide adenine dinucleotide carrier protein

carrier gene has a closer genetic relationship with that of *Lycopersicon esculentum*. This implied that researchers

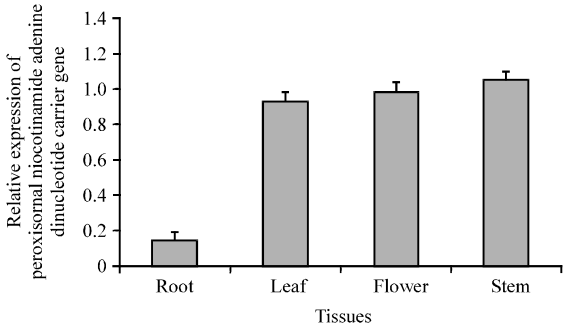

Fig. 6: Expression analysis of peroxisomal nicotinamide adenine dinucleotide carrier gene *mRNA* in various tissues

can use *Lycopersicon esculentum* as model organism to study the tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene or use tobacco as model organism to study the *Lycopersicon esculentum* peroxisomal nicotinamide adenine dinucleotide carrier gene.

From the tissue distribution analysis in the experiment it can be seen that peroxisomal nicotinamide

adenine dinucleotide carrier gene was highly expressed in stem, leaf and flower. For peroxisomal nicotinamide adenine dinucleotide carrier had been characterized to be required for nicotinamide adenine dinucleotide import into peroxisomes, the suitable explanation for this under current conditions is that this transport function is mainly in stem, leaf and flower.

CONCLUSION

Researchers first isolated the tobacco peroxisomal nicotinamide adenine dinucleotide carrier gene and performed necessary sequence analysis and tissue expression profile analysis. These results established the primary foundation of using tobacco nicotinamide adenine dinucleotide as drug for animals and humans in the future.

REFERENCES

Agrimi, G., A. Russo, C.L. Pierri and F. Palmieri, 2012. The peroxisomal NAD^+ carrier of *Arabidopsis thaliana*transports coenzyme A and its derivatives. J. Bioenergy Biomembr., 44: 333-340.

Bernhardt, K., S. Wilkinson, A.P.M. Weber and N. Linka, 2012. A peroxisomal carrier delivers NAD^+ and contributes to optimal fatty acid degradation during storage oil mobilization. Plant J., 69: 1-13.

Ding, D., W. Qi, D. Yu, H. Jiang and C. Han *et al*., 2013. Addition of exogenous NAD^+ prevents mefloquine-induced neuroaxonal and hair cell degeneration through reduction of caspase-3-mediated apoptosis in cochlear organotypic cultures. PloS ONE, Vol. 8. 10.1371/journal.pone.0079817.

Fischer, L.R., D.G. Culver, P. Tennant, A.A. Davis and M. Wang *et al*., 2004. Amyotrophic lateral sclerosis is a distal axonopathy: Evidence in mice and man. Exp. Neurol., 185: 232-240.

Hardison, R.C., 2003. Comparative genomics. PLoS Biol., Vol. 1, No. 2. 10.1371/journal.pbio.0000058.

Hipkiss, A.R., 2010. Aging, proteotoxicity, mitochondria, glycation, NAD^+ and carnosine: Possible inter-relationships and resolution of the oxygen paradox. Front. Aging Neurosci., Vol. 2. 10.3389/fnagi.2010.00010.

Kaneko, S., J. Wang, M. Kaneko, G. Yiu and J.M. Hurrell *et al*., 2006. Protecting axonal degeneration by increasing nicotinamide adenine dinucleotide levels in experimental autoimmune encephalomyelitis models. J. Neurosci., 26: 9794-9804.

Kawai, Y., T. Nakao, N. Kunimura, Y. Kohda and M. Gemba, 2006. Relationship of intracellular calcium and oxygen radicals to Cisplatin-related renal cell injury. J. Pharmacol. Sci., 100: 65-72.

Klaidman, L., M. Morales, S. Kem, J. Yang, M.L. Chang and J.D. Adams Jr., 2003. Nicotinamide offers multiple protective mechanisms in stroke as a precursor for NAD^+, as a PARP inhibitor and by partial restoration of mitochondrial function. Pharmacologist, 69: 150-157.

Liu, G.Y., 2009. Isolation, sequence identification and tissue expression profile of two novel soybean (*Glycine max*) genes-vestitone reductase and chalcone reductase. Mol. Biol. Rep., 36: 1991-1994.

Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_T$ Method. Methods, 25: 402-408.

Luo, J., A.Y. Nikolaev, S.I. Imai, D. Chen, F. Su *et al*., 2001. Negative control of p53 by Sir2α promotes cell survival under stress. Cell, 107: 137-148.

Mano, S., C. Nakamori, Y. Fukao, M. Araki, A. Matsuda, M. Kondo and M.A. Nishimura, 2011. A defect of peroxisomal membrane protein 38 causes enlargement of peroxisomes. Plant Cell Physiol., 52: 2157-2172.

Raff, M.C., A.V. Whitmore and J.T. Finn, 2002. Axonal self-destruction and neurodegeneration. Science, 296: 868-871.

Sadanaga-Akiyoshi, F., H. Yao, S. Tanuma, T. Nakahara and J.S. Hong *et al*., 2003. Nicotinamide attenuates focal ischemic brain injury in rats: With special reference to changes in nicotinamide and NAD^+ levels in ischemic core and penumbra. Neurochem. Res., 28: 1227-1234.

Sasaki, Y., T. Araki and J. Milbrandt, 2006. Stimulation of nicotinamide adenine dinucleotide biosynthetic pathways delays axonal degeneration after axotomy. J. Neurosci., 26: 8484-8491.

Stokin, G.B., C. Lillo, T.L. Falzone, R.G. Brusch and E. Rockenstein *et al*., 2005. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science, 307: 1282-1288.

Zhang, J.G. and W.E. Lindup, 1996. Differential effects of cisplatin on the production of NADH-dependent superoxide and the activity of antioxidant enzymes in rat renal cortical slices in vitro. Pharmacol. Toxicol., 79: 191-198.