Conserved Motifs Identification of Nad1 Protein in 25 Species of Vascular Plants

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***Abstract*—Pathogenic fungi have become a serious threat to agriculture. A wide variety of organisms produce antifungal peptides as part of their innate immunity arsenal. Nad1 is one of subunits of respiratory Complex I (NADH dehydrogenase) in plants which is coded in the mitochondrial genome. NaD1 is a potent antifungal defensin that accumulates in different parts of the plants and its function is to protect the organs from damage by fungal pathogens. In this study, the Nad1 mitochondrial protein reference sequences (RefSeq) belonging to different orders of vascular plant species were downloaded from the National Center for Biotechnology Information (NCBI) in FASTA format. The motifs of protein sequences were found using the program of Multiple Em for Motif Elicitation (MEME; version5.3.1) and Motif Alignment and Search Tool (MAST; version5.3.1) at website (https://meme-suite.org). The parameters of MEME analyses were applied as follows: minimum width for each motif, twenty maximum width for each motif, thirty; maximum number of motifs to find, eight and number of repetitions, zero or one per sequence. Based on the results, all motifs, were common in all sample plants. species All motifs width were 30 but motif 8 which was 20 residues with E-value 7.9e-268. Motif 5 had the less E-value among all motifs which was 1.5e-587. These common conserved motifs among different vascular seed plant species confirmed Nad1 protein functional similarities.**

Motif 5: RMAPVATFMLSLVARAVVPFDYGMVLSDPN

Motif 8: VPAEILGIILPLLLGVAFLV

***Keywords*— Bioinformatics; Conserved motifs; NAD1; Vascular plants.**

I. Introduction

Plants, insects and animals produce a large arsenal of cationic peptides as the first line of defense against potential bacterial and fungal pathogens [1]. Among them plants have developed a number of mechanisms for self-defense, including both physical and chemical factors. These include the rapid reinforcement of cell walls and the production of secondary metabolites (e.g. phytoalexins) and defense-related proteins [2]. Various function by disrupting the cell membrane of the target pathogen leading to leakage of cellular contents and cell death [3]. Though, the mechanism of membrane disruption varies among exclusive antimicrobial molecules and is not constantly properly understood. A wide variety mechanisms for membrane disruption have been proposed. These include: coating the surface and destabilizing the membrane (carpet model), insertion into the membrane and formation of discrete pores by self-association (toroidal pore model) or association with membrane lipids (barrel-stave pore model) [4].

Defense-related proteins are typically small, basic, cysteine-rich molecules with antimicrobial activity. They are distributed widely throughout the plant kingdom and, on the basis of their primary structure, can be grouped into several families [5]. It is described that a new mechanism of membrane destabilization by the plant defensin NaD1 from the ornamental tobacco Nicotiana alata [6]. So, NaD1 is an effective antifungal defensin that accumulates in the flowers of the ornamental tobacco plant Nicotiana alata, in which it functions to protect the reproductive organs from damage by fungal pathogens [7]. NaD1 has a well characterized structure, and numerous features of its mechanism of action have been well described but not entirely elucidated [8]. NaD1 has at least a three-step mechanism of action that involves: interaction with the fungal cell wall [9], movement across the plasma membrane, induction of oxidative stress, and interaction with phosphatidylinositol 4,5 bisphosphate. These processes lead to damage of the inner leaflet of the cell membrane and cell death within 10 min of exposure to NaD1 [6, 10, 11]. The objective of the current study was therefore to characterize the motifs of Nad1 proteins in some plant species.

II. Material and Methods

A. Nad1 protein sequence retrieval

The Nad1 protein Reference Sequences (RefSeq) belonging to different vascular plants species were retrieved from NCBI (http:// www.ncbi.nlm.nih.gov) in FASTA format (date received: Apr 2022). Among the plants sequences, those mitochondrion with the range of 300–600 amino acids were selected by the NCBI filters. Due to the large number of sequences available for nad1 in plants, it is not possible to analyze all the sequences. So, one sequence from each family were downloaded for further analyses. Total data consisted of 25 plants which are listed in Table 1 with their family and accession numbers.

B. Conserved motifs analyses

The motifs of protein sequences were created using the program MEME (Multiple Em for Motif Elicitation; version 5.3.1) and MAST (Motif Alignment and Search Tool; version 5.3.1) at website http://meme-suite.org/ [12] to study the variation of Nad1 in twenty-five different vascular plant species. Compared to other related tools, MEME is able to identify

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smaller individual and more divergent. The parameters of MEME analyses were applied as follows: minimum width for each motif, twenty; maximum width for each motif, thirty; maximum number of motifs to find, eight; and number of repetitions, zero or one per sequence.

TABLE I. THE LIST OF SOME PLANT SPECIES OF NAD1 PROTEIN ANALYZED IN CURRENT STUDY

Index	Scientific name of the plant	Family	Order	Abbreviation	Accession number
1	*Allium cepa*	Amaryllidaceae	Asparagales	*Ac-Nad1*	YP_009252179
2	*Arabidopsis thaliana*	Brassicaceae	Brassicales	*At-Nad1*	YP_009472104
3	*Carica papaya*	Caricaceae	Brassicales	*Cp-Nad1*	YP_002608183
4	*Cocos nucifera*	Arecaceae	Arecales	*Cn-Nad1*	YP_009315934
5	*Dorcoceras hygrometricum*	Gesneriaceae	Lamiales	*Dh-Nad1*	YP_005090403
6	*Eucalyptus grandis*	Myrtaceae	Myrtales	*Eg-Nad1*	YP_009543631
7	*Geranium maderense*	Geraniaceae	Geraniales	*Gm-Nad1*	YP_009137018
8	*Hibiscus cannabinus*	Malvaceae	Malvales	*Hc-Nad1*	YP_009412588
9	*Hyoscyamus niger*	Solanaceae	Solanales	*Hn-Nad1*	YP_009121955
10	*Ipomoea nil*	Convolvulaceae	Solanales	*In-Nad1*	YP_009295069
11	*Lactuca sativa*	Asteraceae	Asterales	*Ls-Nad1*	YP_009652409
12	*Magnolia biondii*	Magnoliaceae	Magnoliales	*Mb-Nad1*	YP_009871044
13	*Nymphaea hybrid cultivar*	Nymphaeaceae	Nymphaeales	*Nh-Nad1*	YP_010250656
14	*Ophioglossum californicum*	Ophioglossaceae	Ophioglossales	*Oc-Nad1*	YP_009277425
15	*Osmanthus fragrans*	Oleaceae	Lamiales	*Of-Nad1*	YP_010249799
16	*Phaseolus vulgaris*	Fabaceae	Fabales	*Pv-Nad1*	YP_009709881
17	*Phlegmariurus squarrosus*	Lycopodiaceae	Lycopodiales	*Ps-Nad1*	YP_006234292
18	*Psilotum nudum*	Psilotaceae	Psilotales	*Pn-Nad1*	YP_009277446
19	*Ricinus communis*	Euphorbiaceae	Malpighiales	*Rc-Nad1*	YP_004237246
20	*Salvia miltiorrhiza*	Lamiaceae	Lamiales	*Sm-Nad1*	YP_008992266
21	*Tolypanthus maclurei*	Loranthaceae	Santalales	*Tm-Nad1*	YP_010137136
22	*Triticum aestivum*	Poaceae	Poales	*Ta-Nad1*	YP_009433707
23	*Utricularia reniformis*	Lentibulariaceae	Lamiales	*Ur-Nad1*	YP_009380834
24	*Vaccinium macrocarpon*	Ericaceae	Ericales	*Vm-Nad1*	YP_008999604
25	*Ziziphus jujuba*	Rhamnaceae	Rosales	*Zj-Nad1*	YP_009241651

III. RESULTS AND DISCUSSION

In a group of protein-related sequences, a sequence pattern is observed repeatedly known as a conserved motif. MEME represents motifs as position-dependent letter-probability matrices, which assign the probability of each possible letter at each position in the pattern, though motifs in MAST are characterized as position-dependent scoring matrices and define the score of each possible letter at each position in the pattern. Eight conserved motifs of each Nad1 family were identified by MEME (Figure 1) which their scores, E-values and the number of motifs' species are listed in Table 2.

TABLE II. CHARACTERIZATION OF EIGHT MOTIFS OF DIFFERENT NAD1 VASCULAR PLANTS SPECIES ANALYZED IN THIS STUDY

Motifs	Width	E-value	Sites	Sequence
Motif 1	30	5.2e-628	25	FISRLAETNRAPFDLPEAEAESVAGYNVEY
Motif 2	30	6.5e-626	25	TVLICVGPRNSSEIVMAQKQIWSGIPLFPV
Motif 3	30	3.0e-615	25	AERKVMAFVQRRKGPDVVGSFGLLQPLADG
Motif 4	30	2.6e-612	25	YIWVRAAFPRYRYDQLMGLGRKVFLPLSLA
Motif 5	30	1.5e-587	25	RMAPVATFMLSLVARAVVPFDYGMVLSDPN
Motif 6	30	7.9e-564	25	YGIIIAGRSSNSKYAFLGALRSAAQMVPYE
Motif 7	30	3.4e-549	25	CTSLSPGGWPPILDLPISKKIPGSIWFSIK
Motif 8	20	7.9e-268	25	VPAEILGIILPLLLGVAFLV

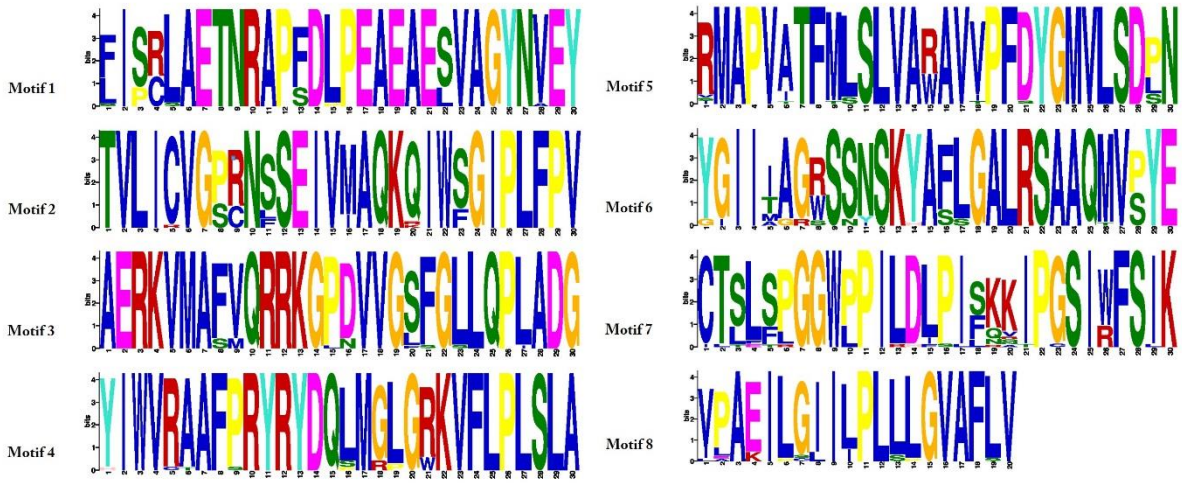

Fig. 1. Sequence logo of Nad1 protein family motif created by MEME.

These findings also showed that all vascular plant species shared eight identical motifs (Figure 2).

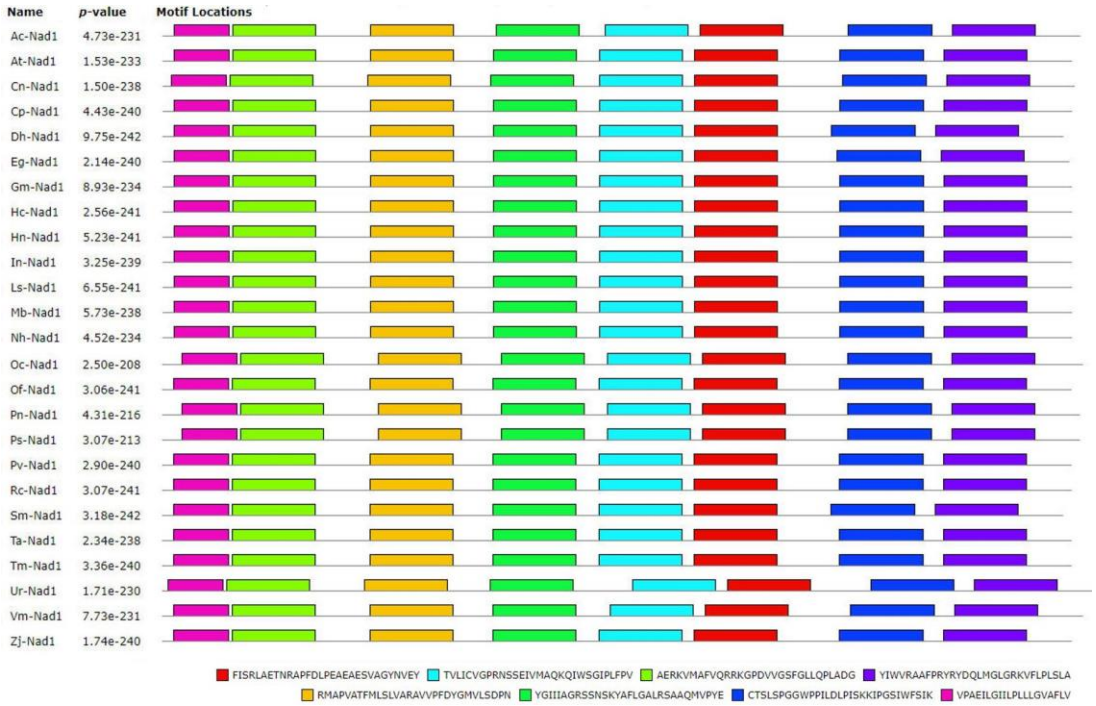

Fig. 2. Motifs of Nad1 protein in vascular plant species. The MEME motifs are shown as different boxes. The p-value of a sequence is the expected number of sequences in a random database of the same size that would match the motifs as well as the sequence.

Nucleotide and protein sequences comprise patterns or motifs that have been preserved through evolution since they are crucial to the function or structure of the molecule. In proteins, these conserved sequences may be involved in the binding of the protein to its substrate or to another protein, may contain the active site of an enzyme or may determine the three dimensional structure of the protein [13]. Members of protein families are often characterized by more than one motif (on average each family has 3-4 conserved regions) which increases the certainty that a protein has been assigned to a precise family [14]. Therefore, motif analysis of Nad1 protein was done and 8 motifs were recognized in this protein family. All motifs were shared in all vascular plant species. Motif 5 had the less E-value among all motifs which was 1.5e-587

CONCLUSION

These common conserved motifs among different vascular seed plant species confirmed Nad1 protein functional similarities.

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