

Genome-Wide Analysis of the DNA-Binding One Zinc Finger (Dof) Family in Vigna Species: Unveiling Insights into Transcriptional Regulation

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The Dof (DNA-Binding One Zinc Finger) transcription factor family is unique to plants and performs various functions associated with activities unique to plants, including defense mechanisms, light and phytohormone responses, and seed development and germination. Though genome-wide studies have been carried out for this family in many species, but there is scarce information about Dof genes in *Vigna* species. In the current study, 79 non-redundant Dof proteins including 35 of them were found in *V. angularis*, 33 in *V. radiata*, and 11 in *V. unguiculata*. Furthermore, a comparative study was conducted on 33 non-redundant Dof proteins found in *Arabidopsis thaliana*. All of the identified Dof proteins in the *Vigna* species were found in the nucleus, according to predicted subcellular localization, which is consistent with the Dof proteins' known function as transcription factors. Moreover, All three *Vigna* species have several Dof proteins with predicted nuclear localization signals, suggesting a possible function for these proteins in nuclear transport and localization. Most Dof proteins were shown to have no introns by gene structure analysis; however, a small number of genes have one or two introns. The motif distribution analysis revealed motifs that were preserved over various clusters, with certain motifs being more common in particular clusters. A phylogenetic tree was constructed to assess the evolutionary hierarchy between the Dof proteins of *Arabidopsis thaliana* and *Vigna* species that revealed four major clusters, with cluster I having the greatest number of Dof proteins. These results could contribute to a better understanding of and roles of Dof genes in *Vigna* species.

Keywords: Genome-wide analysis, *Vigna* species, transcription factor, DNA binding one zinc finger (DOF), Plant genetics, Gene expression.

INTRODUCTION

Beans or pulses are considered the source of human and animal diets having high nutritional value including high protein, carbohydrates, vitamins, minerals, and health-benefiting fatty acids. Moreover, Pulse flour is an excellent candidate for blending due to the high quantities of lysine present in pulse protein and the high amount of folate it contains. Pulses also include a variety of antioxidants that improve health, such as phenolic chemicals and carotenoids. There is a notable concentration of antioxidants (phenolic acid, polyphenols, flavonoids, and tannins) in pulses. Beans belong to the *Vigna* genus which has flowering plants in the *Fabaceae* family (Hall *et al.*, 2017).

The genus *Vigna* consists of approximately 200 species having pantropical distribution, native to tropical regions worldwide (Habib, 2023; Siddiq and Uebersax, 2022). Several species including mung bean (*Vigna radiata*), cowpea

(*Vigna unguiculata*), mat bean (*Vigna aconitifolia*), urd bean (*Vigna mungo*), rice bean (*Vigna umbellata*), and adzuki bean (*Vigna angularis*) of *Vigna* genus are considered potential food crops for millions of people in developing countries and these *Vigna* species exhibited considerable economic importance in recent decades (Harouna *et al.*, 2018). However, these valuable *Vigna* species are vulnerable to many environmental stresses as well as biological stresses. Environmental stress includes extreme temperature (cold or heat), drought, salinity, water-loggings, and oxidative stresses (Harouna, 2020). Biological factors involve many living organisms like bacteria, fungi, parasites, and insects that cause particular diseases in beans (HanumanthaRao *et al.*, 2016).

Genes that respond to these stresses have a vital role in plant physiology (Tutlani *et al.*, 2022). However, these genes are controlled by several transcription factors that regulate their functioning in particular stressed environments. Transcription

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factors (TFs) are the specific proteins that control RNA polymerase activity either by triggering or hindering (Srivastava *et al.*, 2018). These factors attach to the regulatory sequences within the promoter part of the target gene and regulate its expression. These regulatory sequences can act as either repressors or enhancers. A transcriptional factor of plants consists of DNA-binding domain (DBS) that binds to a particular sequence of a target gene, activation domain (AD) that have the binding regions for transcription co-regulators, an oligomerization site, and signal sensing domain (SSD) that can sense any external signal (a signal that can interrupt gene expression) (Wang *et al.*, 2021).

TFs are also classified based on different families such as eukaryotic-specific bZIP, MYB or bHLH factors, and kingdom specific NAC, WRKY, DOF, and DREB factors (Gupta *et al.*, 2015). The concept of the functional and structural relationship between the TFs and the target gene will be helpful in understanding the molecular analysis of the target gene expression. In plants, these transcriptional factors control the gene expression under different environmental and biotic stresses (Weidemüller *et al.*, 2021). Among these, DNA-binding with one zinc finger (DOF) is particularly linked to all plant species and plays its part in gene regulation. The DOF family is considered as one of the highly effective TF-family in plant crops that regulates their normal growth, differentiation, and developmental processes. These factors also control ongoing biological processes like photosynthesis, transpiration, accumulation (seed storage), transportation, germination, flowering duration, light-based gene expression, dormancy, phytohormonal response, and a lot of others that are specifically associated with the plants (Zou and Sun, 2023). DOF genes are extensively distributed throughout the plant genome (Gupta *et al.*, 2015).

The DOF transcriptional factors in *Vigna* species play a vital part in development, growth as well as in conferring resilience to different environmental and biological stresses. A number of DOF transcriptional factors have been reported in *Vigna* species such as WRKY, MYB, and PHD (Li *et al.*, 2023), AP2/ERF (Chen *et al.*, 2022) in mungbean and bZIP in adzuki bean (Yin *et al.*, 2022). Furthermore, the discovery and characterization of DOF genes in *Vigna* species have been made easier by the availability of innovative genomic resources such whole-genome sequencing and high-throughput transcriptome data. By combining these genetic resources with functional investigations, such as functional validation experiments and gene expression profiles, we may improve our comprehension of the biological relevance of the DOF family in *Vigna* species (Chen *et al.*, 2022). The objective of this research study is the genome-wide investigation of DNA binding with one zinc finger (DOF) family in *Vigna* species to thoroughly explore and comprehend the genetic architecture, distribution, and functional functions of DOF family members throughout the

genome of *Vigna* species and the role of transcription factors in plant physiology.

MATERIALS AND METHODS

The DNA-binding one zinc finger (DOF) is an important transcriptional factor present specifically in plants. These DOF factors play vital role in plant-linked cellular functions such as plant growth, differentiation, development as well as their response during biotic and abiotic stresses. Although genome wide analysis of Dof protein family has been explored in many crop species but in case of *Vigna* species, no relevant study has been emerged yet. The present study was conducted to identify the Dof genes, their location on chromosomes, intron-exon architecture and motif distribution as well as comparative evolutionary relationship of non-redundant Dof proteins among *Vigna* species in reference to *Arabidopsis* using computational biology.

Sequence retrieval: A database specify to plant transcription factors, known as PlantTFDB (Plant Transcription Factor database) version 5.0 (<http://planttfdb.gao-lab.org/>) was used for obtaining DOF proteins sequences and CDS in three *Vigna* species (*Vigna angularis*, *Vigna radiata* and *Vigna unguiculata*) and *Arabidopsis thaliana*. However, genomic sequences of Dof protein of three *Vigna* species and *Arabidopsis* were searched from NCBI (National Center for Biotechnology Information) (<https://www.ncbi.nlm.nih.gov/>) database.

Conserved Domain/ motif analysis: Online web servers were used to identify conserved Dof domains among collected Dof protein sequences. Retrieved sequences of Dof proteins were subjected to ScanProsite tool (<https://prosite.expasy.org/scanprosite/>) and Batch Web-CD search tool (<https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>) for identification of Dofdomains. While the conserved motifs within these retrieved protein sequences were identified using MEME (Multiple Expression motifs for Motif Elicitation) search tool (<http://meme-suite.org/tools/meme>). In MEME, maximum number of motif was set at 15 while the remaining parameters were at their default setting.

Physicochemical properties: Online protein servers, ProtParam (<https://web.expasy.org/protparam/>) and PlantTFDB v5.0 were used for collecting information about amino acid composition, isoelectric point (*pI*), molecular weight, molecular formula, instability index (II), aliphatic index and grand average of hydropathicity (GRAVY) of Dof proteins along with chromosomal location of the DOF genes of *Vigna* species.

Protein subcellular localization: The prediction of subcellular localization of DOF protein of three *Vigna* species was performed using CELLO v.2.5 (<http://cello.life.nctu.edu.tw/>). This online server provides



location of Dof proteins within cell based on SVMs (support vector machines) classification system.

Nuclear localization signals (NLS): NLStradamus (<http://www.moseslab.csb.utoronto.ca/NLStradamus/>) was used for predicting NLS regions in investigated Dof proteins of *Vigna* species. This NLS signal prediction was based on hidden markov model.

Gene Structure analysis: In gene structure display analysis, gene sequences were retrieved from their respective selected Dof proteins. The genes structure with intron-exon architecture of retrieved DOF genes was explored through Gene structure display server 2.0 (GDS) (<http://gsds.cbi.pku.edu.cn/>).

Phylogenetic analysis: The amino acid sequences of Dof proteins from three *Vigna* species in reference to Arabidopsis Dof proteins were aligned using CLUSTALW program (<https://www.genome.jp/tools-bin/clustalw>). The Phylogenetic trees were generated using Molecular Evolutionary Genetics Analysis v. 7.0 (MEGA7) software package (Kumar et al., 2016) under neighbor-joining algorithm.

RESULTS

The genome wide analysis is the characterization of genes linked to particular cellular activities in plant species. Although genome wide analysis of Dof family has been explored in many crop species in the case of *Vigna* species, no relevant study has emerged yet. The present study was conducted to identify the Dof genes, their location on chromosomes, intron-exon architecture, and motif distribution as well as the comparative evolutionary

relationship of non-redundant Dof proteins among *Vigna* species and Arabidopsis using computational biology. So, this research study was aimed “In silico Genome-wide probing and analysis of DOF family in *Vigna* species (*Vigna radiata*, *Vigna angularis*, *Vigna unguiculata*) for the characterization and function prediction”.

Genome wide identification of *Vigna* Dof proteins: In genome wide analysis, a total of 79 non redundant Dof proteins in *Vigna* species (35 in *Vigna angularis*, 33 in *Vigna radiata* and 11 in *Vigna unguiculata*) and 33 non redundant Dof proteins in Arabidopsis thaliana were identified from Plant Transcription Factor Database (PlantTFDB) and National Center for Biotechnology Information (NCBI).

Conserved Domain/ motif analysis: The conserved Dof domains were confirmed in 79 non-redundant Dof proteins of three *Vigna* species in BatchCD search and ScanProsite. Moreover, fifteen (15) conserved motifs with different architecture were identified in three *Vigna* species using MEME search tool (Table 1, Figure 1, 2 & 3).

Physicochemical properties of DOF proteins: The physicochemical properties of DOF proteins of three *Vigna* species have been explored. In *V. angularis* Dofs, serine (Ser) was found as a highly abundant amino acid followed by leucine (Leu), glycine (Gly) and arginine (Arg). In *V. angularis*, the length of amino acids for Dof proteins varied from 129 for VangDOF_24 to 516 for VangDOF_16. The average length of amino acids was 310. The maximum molecular weight (MW) for Dof proteins in VangDOF_16 was 56843.80 Da and the minimum molecular weight in VangDOF_24 was 14311.07 Da. The average MW for Dof proteins was 33917.82 Da. The theoretical isoelectric point (pH value where net charge on protein is zero) ranged from

Table 1. Conserved motif in identified Dof proteins of *Vigna* species, predicted by MEME suit.

Motif #	Protein motif of <i>V. angularis</i>	Protein motif of <i>V. radiata</i>	Protein motif of <i>V. unguiculata</i>
1	PPPEQALKPCRCBSTNTKFYYNNYSLSQPRH FCKNCR RYWTGGGALRNV	PKPDEALKPCRCBSTNTKFY YNNYSLSQPRHFCKNCRRYW	CPRCDSTNTKFYYNNYSLSQPRHFCKS CRRYWTGGGTLRNV VGGGCRK
2	PVGGGCRKNKR	TKGGTLRNV PVGGGCRKNKRSSSSSSS	VPKTLRIDDPNEAAKSSIWTTLGKNEVM
3	NGCVLVPKTLRIDDPNEAAKSSIWTTLGKNE	REGCVLVPKTLRIDDPNEAAKSSIWTT LGKNE	GLSSKQVSNRGLDWGQTLLQAQNLPLK PTPMRKQQQQTQ
4	KNRVVEASPVQLQANPAALSRSLNFHESS	EKNRVVEASPVQLQANPAALSRSLNFHESS	NDRLDFGDGSFZQDYDVGSDDLJVN PQI
5	KDPAIKLFGRTIPLP	KDPAIKLFGRTIPLP	MHHSSNTPGFLDSLRSGLGTQSNVQNL Y YGYGNGDMGEVD
6	CSGPNSTPLGKHSRDGBIAKE	PSSSTSSSPCSGPNSTPLGKHSRDGBIAK	KRPKIDQPSVAQMVSVEIQPGNHQPFKNV QENNDFVGSFGA
7	FGSDTPLCESMASVLNLAEK	LTFGPDTPLCESMASVLNLAEK	VERKPRPEPDQALK
8	LASSIESLSSINQDLHWKLQQQLATLFG	NDRLDFGDGSFZQDYDVGSDDLJVN PQI	RDPAIKLFGKTIPF
9	VPWPYPWNPTG	IRPGSMADRARLAKI	MDNLNVFANEDNQVNDVKPPP
10	SHYRHITISEALQPA	VSQTASVKMEE	RVLWGFPWQM
11	NDRLDFGDGSFZQDYDVGSDDLJVN PQI	VPWPYPWN	MDGELRTRVTPPLAVDDHKP TPSSLYQSLCLPQQNPTSTKVSE GKDFGI ETSPVLQANPAALSRSLNFHEQ M
12	KIDQPSVAQMVSVEIQPGN HQPFKNVQENNDFVGSFG ASSS	DHHHHHLM	
13	IRPGSMADRARLANI	RHFCKTCRRYW	RAGSMAERARLANIPLPEAAL
14	VSQTASVKMEEN	RVLFPFEDLK	DLTALKKPKDIJP
15	WHAATASHGGFRHDFPVKRLRCYSDGQSC	QMVSVVEIQPGNHQPFKNVQENNDFVGSF	DHHHHHLM



Table 2. Physicochemical properties of identified *Vigna angularis* Dof proteins and chromosomal location of respected gene.

Sr.	Sequence code	AA	MW (Da)	pI	Molecular formula	II	Aliphatic index	GRAVY	Chromosome
1	VangDOF_1	277	30182.79	9.63	C ₁₃₀₄ H ₂₀₃₁ N ₃₉₇ O ₄₀₂ S ₁₅	61.11	57.40	-0.619	6
2	VangDOF_2	235	25806.56	4.90	C ₁₀₉₆ H ₁₆₉₄ N ₃₁₈ O ₃₆₇ S ₁₉	52.75	56.89	-0.676	6
3	VangDOF_3	272	29604.28	9.78	C ₁₂₈₅ H ₂₀₀₅ N ₃₉₁ O ₃₈₉ S ₁₄	60.71	58.12	-0.591	7
4	VangDOF_4	341	36776.47	8.68	C ₁₅₉₃ H ₂₄₂₈ N ₄₆₈ O ₅₁₂ S ₁₄	54.16	46.36	-0.777	10
5	VangDOF_5	205	21999.59	9.02	C ₉₅₄ H ₁₄₈₁ N ₂₈₅ O ₂₉₆ S ₁₀	55.42	58.54	-0.612	7
6	VangDOF_6	482	52444.96	7.25	C ₂₂₆₁ H ₃₅₂₁ N ₆₅₃ O ₇₄₇ S ₂₀	56.79	52.24	-0.792	10
7	VangDOF_7	207	22280.58	8.70	C ₉₅₅ H ₁₄₈₀ N ₂₈₆ O ₃₁₂ S ₁₀	58.42	51.40	-0.713	9
8	VangDOF_8	269	29555.42	9.49	C ₁₃₀₂ H ₂₀₄₂ N ₃₆₈ O ₃₉₇ S ₁₁	51.43	58.44	-0.687	2
9	VangDOF_9	293	31637.13	6.65	C ₁₃₇₅ H ₂₁₃₀ N ₃₉₄ O ₄₄₂ S ₁₂	53.61	60.65	-0.561	1
10	VangDOF_10	498	54342.74	6.25	C ₂₃₄₃ H ₃₆₀₅ N ₆₇₉ O ₇₇₄ S ₂₁	59.13	48.27	-0.796	1
11	VangDOF_11	443	48115.01	7.82	C ₂₀₆₈ H ₃₂₆₇ N ₆₀₃ O ₆₆₅ S ₂₈	44.14	62.98	-0.540	4
12	VangDOF_12	142	16539.59	8.97	C ₇₂₁ H ₁₁₁₁ N ₂₂₁ O ₂₁₃ S ₈	51.79	45.35	-1.059	1
13	VangDOF_13	300	33431.04	5.49	C ₁₄₄₄ H ₂₂₂₂ N ₄₁₄ O ₄₆₉ S ₁₇	60.11	60.17	-0.633	2
14	VangDOF_14	335	35757.82	8.72	C ₁₅₅₃ H ₂₃₉₄ N ₄₄₈ O ₄₉₂ S ₁₇	53.05	54.81	-0.521	9
15	VangDOF_15	271	29296.61	8.46	C ₁₂₇₄ H ₁₉₈₈ N ₃₅₈ O ₄₁₄ S ₁₁	43.88	59.78	-0.549	1
16	VangDOF_16	516	56843.80	6.77	C ₂₄₆₁ H ₃₈₄₀ N ₆₈₈ O ₇₉₁ S ₃₅	48.17	59.38	-0.597	11
17	VangDOF_17	278	30885.31	8.58	C ₁₃₃₅ H ₂₀₈₁ N ₃₈₅ O ₄₃₄ S ₁₃	50.44	54.35	-0.810	5
18	VangDOF_18	304	32793.12	9.04	C ₁₃₉₆ H ₂₁₉₅ N ₄₂₃ O ₄₆₇ S ₁₃	57.41	56.22	-0.721	5
19	VangDOF_19	306	33198.05	3.09	C ₁₄₄₁ H ₂₂₃₉ N ₄₀₉ O ₄₆₂ S ₁₆	46.04	67.52	-0.416	5
20	VangDOF_20	179	20284.21	9.43	C ₈₇₈ H ₁₃₉₅ N ₂₆₇ O ₂₅₉ S ₁₄	71.40	52.91	-0.733	5
21	VangDOF_21	253	27657.96	8.81	C ₁₂₁₃ H ₁₈₅₇ N ₃₄₅ O ₃₇₃ S ₁₃	49.63	53.60	-0.729	5
22	VangDOF_22	501	54516.29	5.95	C ₂₃₆₅ H ₃₆₈₄ N ₆₆₈ O ₇₈₁ S ₁₇	50.61	55.68	-0.830	4
23	VangDOF_23	307	33379.29	9.15	C ₁₄₄₇ H ₂₂₃₈ N ₄₂₆ O ₄₅₂ S ₁₇	49.28	58.14	-0.570	4
24	VangDOF_24	129	14311.07	9.73	C ₆₁₇ H ₉₆₉ N ₁₉₁ O ₁₈₉ S ₇	47.44	49.84	-0.891	4
25	VangDOF_25	271	29450.12	9.52	C ₁₂₇₀ H ₂₀₁₉ N ₃₈₅ O ₃₉₇ S ₁₃	50.02	62.29	-0.573	4
26	VangDOF_26	225	25209.15	8.64	C ₁₁₀₀ H ₁₆₈₃ N ₃₁₅ O ₃₄₂ S ₁₃	56.34	48.53	-0.814	8
27	VangDOF_27	322	35302.00	5.74	C ₁₅₁₈ H ₂₃₂₅ N ₄₃₉ O ₄₉₆ S ₂₀	48.95	57.24	-0.615	8
28	VangDOF_28	338	36876.19	6.62	C ₁₅₈₀ H ₂₄₂₉ N ₄₆₁ O ₅₀₄ S ₂₉	42.59	52.81	-0.656	2
29	VangDOF_29	400	44327.44	8.75	C ₁₉₀₃ H ₃₀₀₂ N ₅₇₂ O ₆₁₂ S ₂₀	53.33	52.75	-0.932	2
30	VangDOF_30	475	52615.65	7.21	C ₂₂₈₆ H ₃₅₈₇ N ₆₅₅ O ₇₃₇ S ₁₈	43.50	62.36	-0.686	1
31	VangDOF_31	232	24392.98	8.72	C ₁₀₅₂ H ₁₆₀₂ N ₃₁₄ O ₃₃₃ S ₁₃	53.16	45.82	-0.568	1
32	VangDOF_32	278	30206.78	8.77	C ₁₃₂₇ H ₂₀₃₅ N ₃₈₁ O ₄₀₆ S ₁₂	47.91	65.97	-0.440	1
33	VangDOF_33	294	32193.19	8.51	C ₁₃₈₈ H ₂₁₆₇ N ₄₀₅ O ₄₃₇ S ₂₁	46.57	64.97	46.57	1
34	VangDOF_34	232	25277.80	6.48	C ₁₁₀₈ H ₁₆₅₈ N ₃₂₄ O ₃₄₄ S ₈	28.21	57.20	-0.594	1
35	VangDOF_35	457	49632.66	8.40	C ₂₁₇₀ H ₃₃₈₇ N ₆₁₃ O ₆₈₃ S ₂₀	53.26	61.07	-0.613	1

3.09 for VangDOF_19 to 9.78 for VangDOF_3. Most of the VangDOF proteins were in the basic range of pI value with an average of 7.93. The instability index is basically a stability measure of proteins and if the instability index is less than 40 than protein is considered to be stable. The instability index (II) for VangDOF_34 was 28.21, considered stable while the rest of the Dof proteins were unstable. Aliphatic index is the relative volume covered by aliphatic amino acids in the side chain. The aliphatic index ranged from 45.35 for VangDOF_12 to 67.52 for VangDOF_19 with an average of 56.29. GRAVY index measures hydrophathy (hydrophilic-positive GRAVY and hydrophobic-negative GRAVY) value for peptides. The GRAVY value of VangDOFs was in the

negative range. In addition, the chromosomal location of the respected Dof gene was also given in Table 2.

In *Vigna radiata* Dofs, serine (Ser) was found a highly abundant amino acid followed by glycine (Gly), lysine (Lys) and threonine (Thr). In *V. radiata*, the length of amino acids for Dof proteins varied from 156 for VradiDOF_3 to 501 for VradiDOF_11. The average length of amino acids was 308.27. The maximum molecular weight (Da) for Dof proteins in VradiDOF_11 was 54602.38 and the minimum molecular weight (Da) in VradiDOF_3 was 17857.24. The average molecular weight (Da) for Dof protein was 33605.34. The theoretical isoelectric point ranged from 5.62 for VradiDOF_24 to 9.78 for VradiDOF_10. Most of the VradiDOF proteins were in the basic range of pI value with



Table 3. Physicochemical properties of identified *Vigna radiata* Dof proteins and chromosomal location of respected gene.

Sr.	Sequence code	AA	MW (Da)	pI	Molecular formula	II	Aliphatic index	GRAVY	Chromosome
1	VradiDof_1	479	52095.14	6.57	C2229H3531N657O731S27	48.21	61.09	-0.681	4
2	VradiDof_2	253	27711.09	8.81	C1220H1872N344O373S12	49.19	58.04	-0.693	5
3	VradiDof_3	156	17857.24	9.39	C778H1219N239O228S9	41.25	51.92	-0.896	1
4	VradiDof_4	214	23756.47	6.58	C1024H1606N298O332S11	58.59	66.50	-0.581	2
5	VradiDof_5	465	50749.57	6.52	C2164H3387N627O715S35	49.72	51.63	-0.696	11
6	VradiDof_6	307	33258.13	9.53	C1436H2239N437O447S15	61.71	59.12	-0.625	6
7	VradiDof_7	263	29434.68	6.38	C1283H1934N372O397S16	45.47	54.83	-0.666	4
8	VradiDof_8	359	39463.83	8.47	C1696H2656N506O549S17	53.94	49.55	-0.944	2
9	VradiDof_9	193	20691.92	9.57	C879H1375N275O284S11	58.07	47.05	-0.660	4
10	VradiDof_10	272	29565.29	9.78	C1285H2008N388O389S14	63.02	57.76	-0.583	7
11	VradiDof_11	501	54602.38	5.96	C2362H3682N670O783S19	51.45	54.35	-0.849	4
12	VradiDof_12	334	35775.77	6.50	C1545H2404N452O497S16	46.61	63.08	-0.501	4
13	VradiDof_13	338	36941.33	6.62	C1588H2444N460O504S28	43.15	54.85	-0.624	2
14	VradiDof_14	211	23003.88	9.11	C1006H1558N294O305S11	52.52	61.94	-0.542	7
15	VradiDof_15	473	52403.35	8.34	C2274H3571N657O734S17	41.63	61.40	-0.703	1
16	VradiDof_16	456	49360.73	7.24	C2126H3314N616O697S22	60.83	52.48	-0.710	1
17	VradiDof_17	227	24176.59	9.00	C1033H1586N316O335S12	62.93	43.39	-0.707	1
18	VradiDof_18	288	31246.19	8.48	C1364H2117N389O421S17	44.39	65.73	-0.444	1
19	VradiDof_19	329	35862.16	6.81	C1538H2390N448O492S26	50.46	62.19	-0.541	1
20	VradiDof_20	209	22947.50	8.90	C987H1558N288O325S9	47.63	50.43	-0.869	1
21	VradiDof_21	224	24117.66	9.24	C1036H1605N317O331S10	43.77	54.02	-0.779	1
22	VradiDof_22	455	49462.50	8.43	C2166H3373N611O678S20	50.13	58.33	-0.672	1
23	VradiDof_23	250	27139.87	6.02	C1186H1794N338O379S9	38.79	57.36	-0.650	1
24	VradiDof_24	296	32375.59	5.62	C1386H2124N408O458S17	50.99	55.68	-0.686	8
25	VradiDof_25	216	23858.70	9.25	C1034H1600N304O322S13	53.49	46.99	-0.832	8
26	VradiDof_26	207	22141.38	8.70	C948H1467N283O312S10	54.70	49.03	-0.715	9
27	VradiDof_27	320	34249.29	8.14	C1498H2297N423O467S17	52.18	57.66	-0.413	9
28	VradiDof_28	293	32244.59	9.68	C1423H2240N402O430S12	54.13	62.97	-0.612	2

Table 4. Physicochemical properties of identified *Vigna unguiculata* Dof proteins.

Sr.	Sequence code	AA	MW (Da)	pI	Molecular formula	II	Aliphatic index	GRAVY
1	VunDof_1	342	37357.79	6.66	C1603H2468N466O510S29	46.79	53.65	-0.622
2	VunDof_2	333	35601.53	8.62	C1527H2398N444O505S17	60.71	62.10	-0.509
3	VunDof_3	273	30354.54	6.88	C1307H1999N377O426S17	55.95	50.73	-0.792
4	VunDof_4	306	33437.91	6.58	C1420H2195N421O473S22	55.57	50.03	-0.767
5	VunDof_5	277	30698.14	8.57	C1328H2076N378O434S13	53.75	52.74	-0.806
6	VunDof_6	245	26600.78	8.89	C1117H1739N341O370S16	56.99	46.98	-0.833
7	VunDof_7	223	24327.53	9.78	C1033H1638N314O333S10	75.36	54.62	-0.794
8	VunDof_8	222	24451.78	9.80	C1053H1700N316O328S13	60.67	60.23	-0.766
9	VunDof_9	208	23040.17	9.82	C1002H1598N296O306S11	50.92	55.24	-0.786
10	VunDof_10	206	22147.67	9.16	C959H1481N289O298S10	52.51	53.54	-0.690
11	VunDof_11	161	16694.84	9.94	C714H1160N214O231S8	51.95	57.02	-0.442

an average of 8.02. The instability index for VradiDof_23 was 38.79 considered as stable while the rest of the Dof proteins were unstable. The aliphatic index ranged from 43.39 for VradiDof_17 to 66.50 for VradiDof_4 with an average of 55.80, whereas the average GRAVY value was -0.68. In addition, chromosomal location of the respected Dof gene was also given in Table 3.

In *Vigna unguiculata* Dofs, serine (Ser) was found as highly abundant amino acid followed by glycine (Gly) and threonine (Thr). In *V. unguiculata*, the length of amino acids for Dof proteins was varied from 161 for VunDof_11 to 342 for VunDof_1. The average length of amino acids was 254.18.

The maximum molecular weight (Da) for Dof proteins in VunDof_1 was 37357.79 and the minimum molecular weight (Da) in VunDof_11 was 16694.84. The average molecular weight (Da) for *V. unguiculata* Dof proteins was 25192.06. Their theoretical pI values were ranged from 6.58 for VunDof_4 to 9.94 for VunDof_11. Most of the VunDof proteins were in basic range of pI value with an average of 8.61. In *V. unguiculata*, all Dof proteins were unstable with more than 40 instability index. The aliphatic index ranged from 46.98 for VunDof_6 to 62.10 for VunDof_2 with an average of 54.26. The average GRAVY value was -0.17 (Table 4).



Subcellular localization and nuclear localization signal prediction in DOF proteins:

Subcellular localization is the location of proteins that present in plant cells. In general, all Dof proteins are present in nucleus (Kushwahan *et al.*, 2008). Thereby, the subcellular location for Dof proteins of three *Vigna* species has also been predicted. The predicted localization through software program (CELLO 2.5) indicated that all 35 *V. angularis* Dof proteins were found in nucleus with exception of VangDOF_32 which was found both in nucleus and extracellular space. Similarly, subcellular localization prediction of *V. radiata* indicated that all 33 VradiDOF proteins were found in nucleus with exception of VradiDOF_3 which was found both in nucleus and extracellular space. While the predicted localization of *V. unguiculata* indicated that all identified VunDOF proteins were found in nucleus. The nuclear localization signal (NLS) is a particular sequence of amino acids found in proteins. These sequences tag proteins for their transport into the nucleus through specific nuclear channels. These signals consist of positively charged amino acids such as lysine and arginine. NLStradamus predicted the NL sequences in identified DOF proteins of three *Vigna* species. For *V. angularis*, results showed NL sequences in VangDOF_1, VangDOF_DO3, VangDOF_DO15, VangDOF_DO17, VangDOF_DO20 and VangDOF_DO23 proteins (Table 5). While, for *V. radiata* the NL sequence were predicted in VradiDOF_6, VradiDOF_10, VradiDOF_12, VradiDOF_20 and VradiDOF_31 proteins (Table 6). Similarly, for *V. unguiculata* the NL sequence were predicted in VunDOF_5, VunDOF_8 and VunDOF_9 proteins (Table 7).

Table 5. Nuclear localization signal in *Vigna angularis*.

Sr.	Sequence code	NLS signal
1	VangDOF_1	102 - RRNKKNKRSRSK – 113
2	VangDOF_3	91 - RRNKKNKRSRSK – 102
3	VangDOF_15	48 - GGRKNKRVKK – 57
4	VangDOF_17	93 - GGRKNKRAKK – 102
5	VangDOF_20	27 - KGR – 29
6	VangDOF_23	77 - RNKRNKGSGGRSKS – 90

Table 6. Predicted nuclear localization signal (NLS) in *Vigna radiata*.

Sr.	Sequence Code	NLS signals
1	VradiDOF_6	121 - RRNKKNKRSRSK – 132
2	VradiDOF_10	91 - RRNKKNKRSRSK – 102
3	VradiDOF_12	104 - RNKRKSGSGGRSKS – 117
4	VradiDOF_20	99 - GGRKNKRVKK – 108
5	VradiDOF_31	93 - GGRKNKRAKK – 102

Table 7. Predicted nuclear localization signal (NLS) in *Vigna unguiculata*.

Sr.	Sequence Code	NLS signal
1	VunDOF_5	93 - GGRKNKRAKK – 102
2	VunDOF_8	100 - GGRKNKRVKK – 109
3	VunDOF_9	93 - GGRKNKRAKK – 102

Comparative phylogenetic analysis, intron-exon architecture and motif distribution:

In genome wide analysis, a total of 15 conserved motifs were identified using MEME tool and conserved domains were identified using ScanProsite and CD search tool in each *Vigna* species. The pattern of NLS and protein cellular localization was predicted using NLStradamus and CELLO v. 2.5 respectively. A phylogenetic tree was constructed to evaluate the evolutionary relationship among three *Vigna species* in reference to *Arabidopsis thaliana* using their retrieved sequences of Dof proteins using MEGA7 software. This phylogenetic tree was compared to conserved motif distribution in Dof proteins and exon- intron architecture of Dof genes. The comparative study on *V. angularis* and *Arabidopsis* was carried out using their retrieved Dof sequences. The 35 VangDof proteins and 33 AtDof proteins were combined to generate a neighbor joining tree. Their phylogenetic relationship revealed four major clusters (cluster I, II, III, and IV). Among them, the cluster I was comprised of twenty Dof proteins (10 of *V. angularis* and 10 of *Arabidopsis*).

Their gene structure display study revealed all Dof proteins were intronless except VangDOF_11 which had only one intron and VangDOF_29 which had two introns. A maximum number of conserved protein motifs were found in cluster I (motif 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 14 and 15). VangDOF6, 10, 16, and 22 proteins had maximum number of motif (9 motifs) among all other member of this cluster analysis. While, cluster II was the smallest cluster having 6 Dof proteins (4 Dof proteins of *V. angularis* and 2 Dof proteins of *Arabidopsis*). In cluster II, all Dof proteins were intronless except VangDOF_19 which had only one intron. Dof proteins of cluster II had two motifs (motif 1 and 2) while VangDOF_19 and VangDOF_15 had only one motif. Though, cluster III was consisted of 20 Dof proteins (10 of *V. angularis* and 10 of *Arabidopsis*).

Their exon-intron architecture confirmed one (1) intron in VangDOF1, VangDOF23 and, VangDOF34, while two (2) introns in VangDOF25. Motif distribution confirmed motif 1 and motif 2 in all Dof members of this cluster, while motif 14 was also predominant in 9 Dof proteins (Vang_DO1, 14, 23, 25 and AtDOF1.1, 2.2, 5.1, 3.6, 2.4). In addition, motif 13 was found in VangDOF_1, VangDOF_14, AtDOF2.4, AtDOF3.6, AtDOF5.1 and motif 8 was found in VangDOF_4, AtDOF4.7 and AtDOF5.7. However, Cluster IV was also comprised of 20 Dof proteins (9 of *V. angularis* and 11 of *Arabidopsis*). In cluster IV, all Dof proteins were intronless except VangDOF_8, VangDOF_26 and VangDOF_27 that had only one intron. In Cluster IV, motif 1 and motif 2 were found in all Dof proteins. Motif 9 and 10 were only present in 4 *Arabidopsis* Dof proteins (AtDOF2.1, AtDOF3.2, AtDOF5.3 and AtDOF1.8). Whereas VangDOF_2 and VangDOF_9 were seemed to be distinct one in this



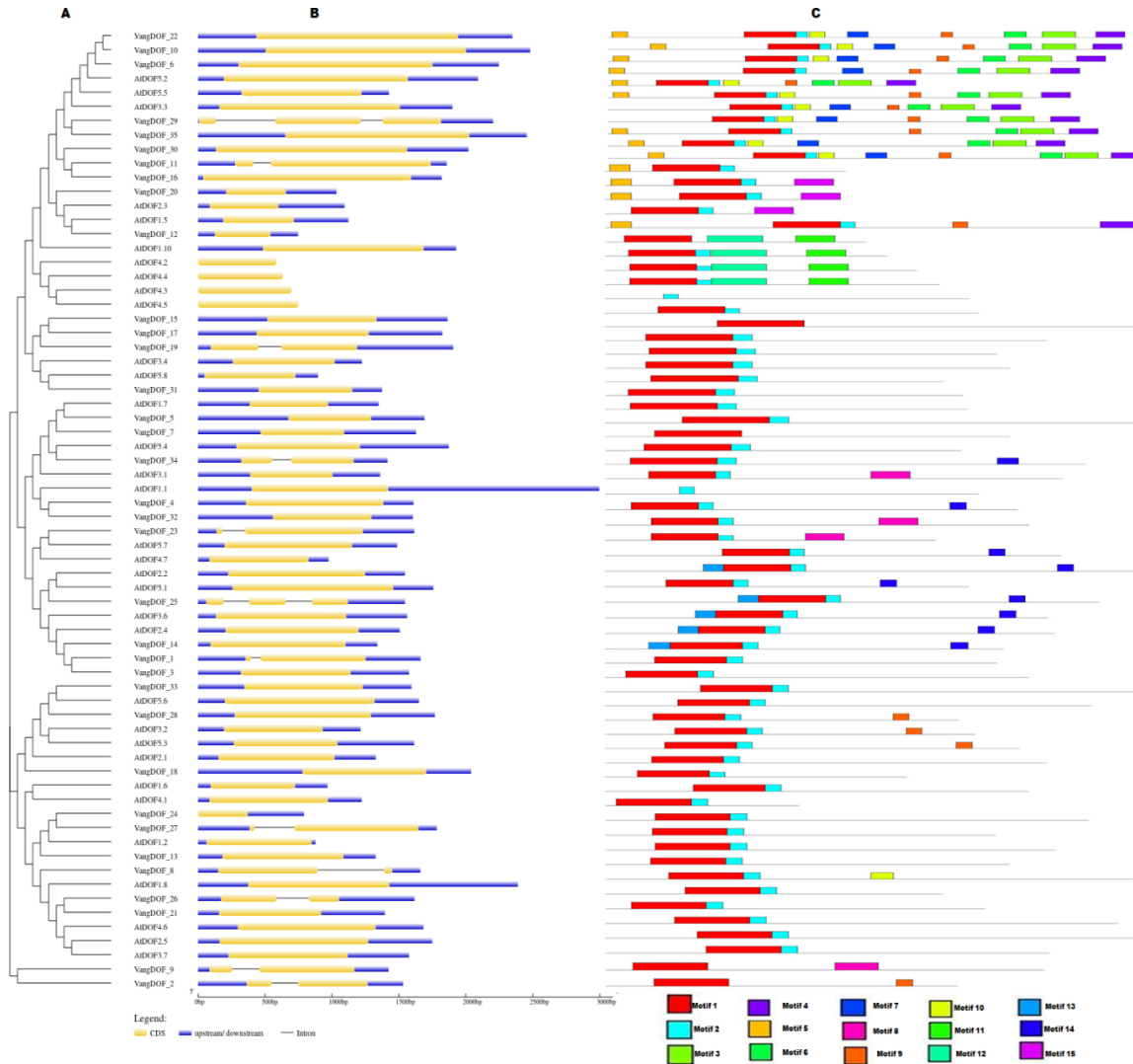


Figure 1. Comparative phylogenetic analysis of *V. angularis* and *Arabidopsis*, gene structure display and conserved motif distribution (A) Phylogenetic tree showed four major Dof clusters. (B) Gene structure analysis indicated that most of the Dof genes from *Vigna* species and *Arabidopsis* were intronless. (C) Several conserved motifs of Dofproteins were shown. These motifs were coded by 15 different colors. Each motif is represented by a specific color.

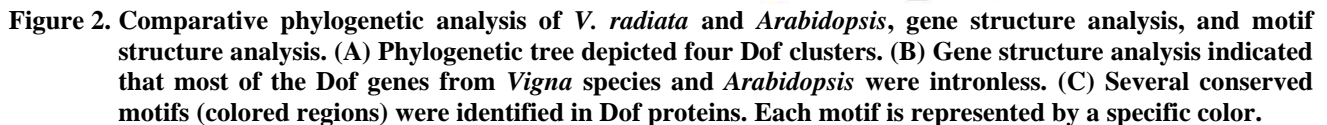
cluster analysis with larger branch length and consisted of two protein motifs (motif 1 and 8 in VangDOF_2, motif 1 and 9 in VangDOF_9) and also have one intron (Figure 1).

The comparative study on *V. radiata* and *Arabidopsis* was carried out using their retrieved Dof sequences. The 33 VradiDof proteins and 33 AtDof proteins were combined to generate a neighbor joining tree. Their comparative phylogenetic relationship depicted four major clusters (cluster I, II, III, and IV). Cluster I was comprised of 21 Dof proteins (11 of *V. radiata* and 10 of *Arabidopsis*).

Their gene structure architecture revealed VradiDOF_9 (2 intron, 3 exon), VradiDOF_21 and VradiDOF_29 (1 intron, 2 exon) have introns while all other Dof member of this cluster

were comprised of exon region only. Their motif distribution revealed five conserved protein motifs (motif 1, 2, 9, 10, 12 and 13) in the member of this clusters. Among these, motif 1 and 2 were found in all Dof members. Moreover, motif 9 was found in VradiDOF_6, 27, AtDOF2.4, 3.6, 5.1, motif 10 VradiDOF_6, 9, 12, 27, AtDOF1.1, 2.2, 2.4, 3.6, 5.1, motif 12 AtDOF 2.2, 5.1 and motif 13 in VradiDOF_23. While cluster II was comprised of 18 Dof proteins (8 of *V. radiata* and 10 of *Arabidopsis*). Their gene structure display revealed VradiDOF_25 have one intron and two exons, however, all other Dof proteins have exon region only. There were 4 conserved protein motifs (motif 1, 2, 11, 12 and 14) in these Dof proteins. Moreover, motif 11 was





with only four Dof proteins of *Arabidopsis* (AtDOF4.2, AtDOF4.3, AtDOF4.4, and AtDOF4.5). In these clustering proteins, motif 1, 2, 8 and 15 were observed. However, in this clustering analysis, VradiDOF_4 and VradiDOF_18 were seemed as distinct and comprised of 2 conserved motifs (motif 2 and 13). The VradiDOF_4 had two exons and one intron, while VradiDOF_18 had only one exon (Figure 2).

The comparative study on *V. unguiculata* and *Arabidopsis* was carried out using their retrieved Dof sequences. The 11 VunDof proteins and 33 AtDof proteins were combined to generate a neighbor joining tree. Their comparative phylogenetic relationship revealed four major clusters (cluster I, II, III, and IV). Among them, cluster I was comprised of 13 Dof proteins (3 of *V. unguiculata* and 10 of *Arabidopsis*).

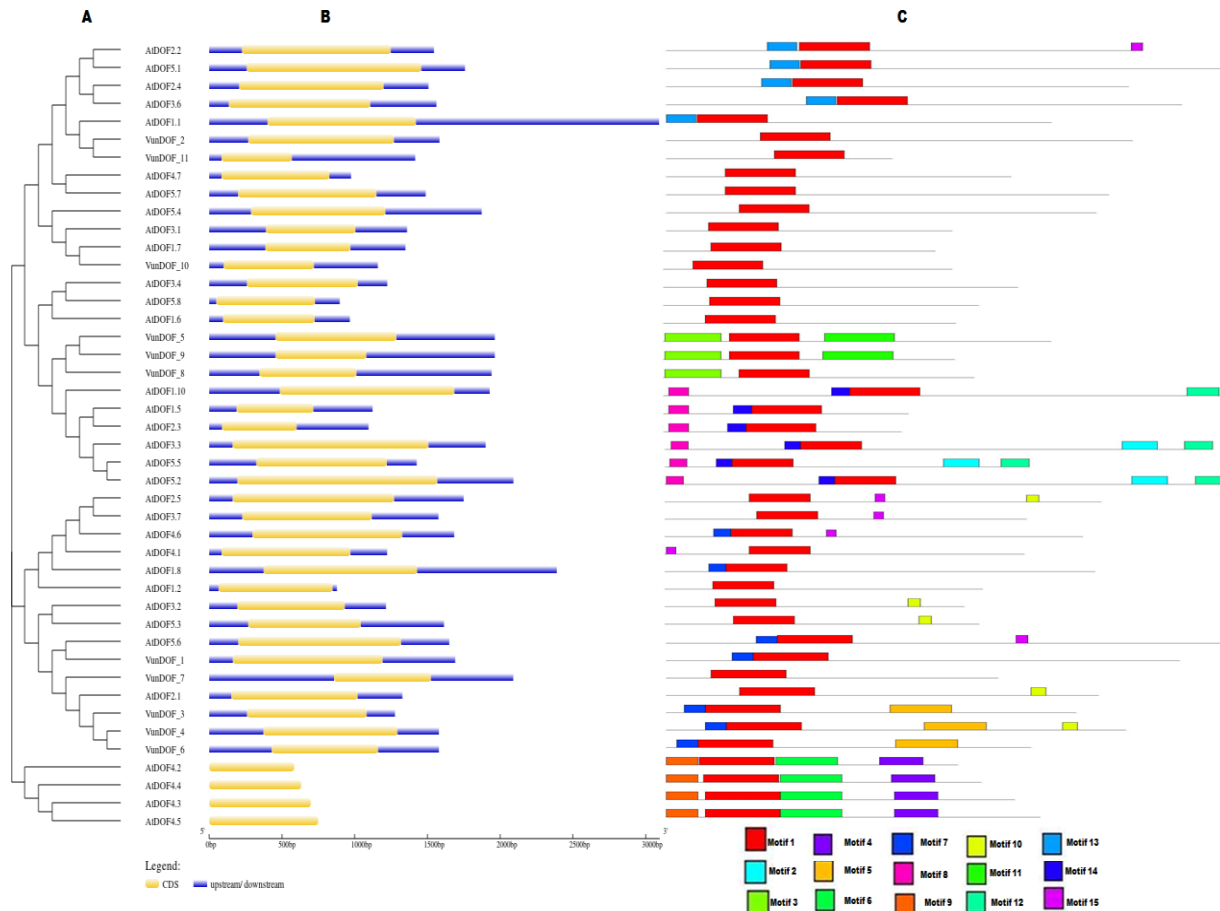


Figure 3. Comparative phylogenetic analysis of *V. unguiculata* and *Arabidopsis*, gene structure display and conserved motif distribution (A) Phylogenetic tree showed four major Dof clusters. (B) Gene structure analysis indicated that all the Dof genes from *Vigna* species and *Arabidopsis* were intronless. (C) Several conserved motifs of Dof proteins were shown. These motifs were coded by 15 different colors.

Their gene structure display study revealed that all Dof members of cluster I had only exon region. Their motif distribution confirmed the presence of motif 1 in all Dof proteins while AtDOF1.1, AtDOF2.2 AtDOF2.4, AtDOF3.6, and AtDOF5.1 had two motifs (motif 1 & 13). Cluster II was consisted of 12 Dof proteins (3 of *V. unguiculata* and 9 of *Arabidopsis*). All Dof members of Cluster II had only exon region. Their motif distribution confirmed the presence of 7 motifs (motif 1, 2, 3, 8, 11, 12, and 14). Cluster III was the largest cluster, having 15 Dof proteins (10 of *V. unguiculata* and 5 of *Arabidopsis*). All Dof members of cluster III had only exon region. There were 5 conserved motifs (motif 1, 5, 7, 10 & 15) of DOF protein member of this cluster, while motif 1 and 7 are predominant followed by motif 10 and 15. Whereas, cluster IV was the smallest group c 4 consisted of Dof proteins of *Arabidopsis* and having four conserved protein motifs (motif1, 3, 4 and 9). Their gene structure display study revealed that all Dof genes were comprised of just exon regions (Figure 3).

DISCUSSION

Plant-specific transcription factors known as the DNA-binding with one finger (DOF) family are important regulators of many physiological and developmental processes, including as seed maturation, germination, blooming, and responses to environmental stresses (Noguero *et al.*, 2013). The highly conserved DOF domain, which is a single zinc finger motif made up of about 50 amino acids, is what distinguishes the DOF proteins from other DNA-binding proteins. DOF proteins may selectively attach to the cis-regulatory elements found in the promoters of their target genes thanks to this pattern, which modifies the expression of those genes (Waqas *et al.*, 2020).

Genome-wide studies of the DOF family have been carried out in several plant species recently in a bid to clarify their evolutionary background, functional diversity, and regulatory networks. Among these species, the *Vigna* genus which



contains significant leguminous crops including adzuki bean (*Vigna angularis*), cowpea (*Vigna unguiculata*), and mung bean (*Vigna radiata*) has attracted a lot of interest because of its importance in agriculture and nutritional value. Gaining knowledge of the DOF gene range in *Vigna* species might help one better understand the molecular mechanisms governing development, growth, and stress responses in these organisms (Zhang *et al.*, 2021).

In current study, a total of 79 non redundant Dof proteins in *Vigna* species (35 in *Vigna angularis*, 33 in *Vigna radiata* and 11 in *Vigna unguiculata*) and 33 non-redundant Dof proteins in *Arabidopsis thaliana* were identified these results were consistent with the previous study that found 30 Dof genes in *Oryza sativa* (Khan *et al.*, 2021), 43 Dof genes in sweet potato (Zhang *et al.*, 2023), and 50 HaDof genes in sunflower (Song *et al.*, 2024). Previous studies found the role of Dof genes in response to exogenous hormones in plants and their role in abiotic stress resilience in sunflower seedlings (Song *et al.*, 2024). The conserved Dof domains were confirmed in 79 non-redundant Dof proteins of three *Vigna* species in BatchCD search and ScanProsite. Moreover, fifteen (15) conserved motifs with different architecture were identified in three *Vigna* species. Moreover, most of the motifs were shared by all three *Vigna* species.

Subcellular localization is the location of proteins that are present in plant cells. In general, all Dof proteins are present in the nucleus (Kushwaha *et al.*, 2008; Gupta *et al.*, 2015; Jiang *et al.*, 2020). Thereby, the subcellular location for Dof proteins of three *Vigna* species has also been predicted. The predicted localization indicated that Dof proteins were found in nucleus except VangDOF_32 and VradiDOF_3 which were found both in the nucleus and extracellular space. These results were validated by previous studies where most of the Dof proteins were found in the nucleus where they play key roles (Lohani *et al.*, 2021; Cao *et al.*, 2022). The nuclear localization signal (NLS) is a particular sequence of amino acids found in proteins. These sequences tag proteins for their transport into the nucleus through specific nuclear channels. These signals consist of positively charged amino acids such as lysine and arginine. NLStradamus predicted the NL sequences in identified DOF proteins of three *Vigna* species. These signals are considered as highly conserved and in directing the localization of the proteins in cell (Alghanem *et al.*, 2023). Moreover, a maximum likelihood-based phylogenetic tree was generated to assess the evolutionary hierarchy of the identified Dof genes.

Conclusion: The objective of this investigation was to discover and characterize the DOF transcription factor, as well as to ascertain the genomic organization, evaluate the evolutionary connections among them, and clarify any possible regulatory roles in *Vigna* species. To better understand plant biology and possibly contribute to the development of improved crop varieties with desired traits,

researchers hope to gain insight into the molecular mechanisms underlying the various biological processes, such as growth, development, stress responses, and metabolism that are regulated by members of the DOF family.

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Ethical statement: This article does not contain any research with human participants or animal performed by any of the authors.

Availability of data and material: We declare that the submitted research article is our own work which has not been published before and is not currently considered for elsewhere publication.

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