

RESEARCH ARTICLE



Genome-wide identification and analysis of the SnRK2 gene family in cowpea (Vigna unguiculata (L.) Walp.) reveals potential stress-responsive roles

Rajesh S1*#, Kavya, S1#, Navinraj S1, Radhamani T1, Anitha T2 & Ramesh S V3

¹Centre for Plant Molecular Biology and Biotechnology (CPMB&B), Tamil Nadu Agricultural University, Coimbatore 641 003. TamilNadu, India ²TNAU-Horticultural College and Research Institute, Periyakulam 625 604, Tamil Nadu, India

³ICAR- Central Plantation Crops Research Institute, Kasaragod 671 124, Kerala, India

#Contributed equally to the work

*Email: rajesh.s@tnau.ac.in

OPEN ACCESS 6

ARTICLE HISTORY

Received: 26 January 2025 Accepted: 18 February 2025 Available online Version 1.0:25 February 2025 Version 2.0: 28 February 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is

available at https://horizonepublishing.com/ journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/ index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an openaccess article distributed under the terms of the Creative Commons Attribution License. which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/ by/4.0/)

CITE THIS ARTICLE

Rajesh S, Kavya S, Navinraj S, Radhamani T, Anitha T, Ramesh SV. Genome-wide identification and analysis of the SnRK2 gene family in cowpea (Vigna unguiculata (L.) Walp.) reveals potential stressresponsive roles. Plant Science Today. 2025; 12(1): 1-6. https://doi.org/10.14719/pst.7431

Abstract

Plants, owing to their sessile nature, have evolved mechanisms to adapt and overcome various abiotic stresses by activating different signaling pathways triggering accumulation of stress-associate proteins. A key regulator in Abscisic acid (ABA) signaling pathway, sucrose non-fermenting-1-related protein kinase 2 (SnRK2), is a plant-specific serine/threonine kinase family involved in osmotic stress responses. While members of this protein family have been analyzed in some plant species, their characterization in cowpea (Vigna unguiculata), a tropical food grain legume cultivated in Africa and Southeast Asia, remains unexplored. Drought stress significantly hampers the growth and productivity of cowpea, highlighting the need for functional studies of stress-related genes. The genes encoding SnRK2 in cowpea and their detailed characterization remain unexplored. The present study attempts to identify and characterize SnRK2 gene families in cowpea using bioinformatics tools. Analysis of the draft genome of Vigna unquiculata in NCBI and Phytozome databases revealed sixteen SnRK2 genes. In silico analysis were conducted to determine gene structure, transcript length and chromosomal mapping of the genes to Vigna unquiculata genome. Domain architectures of the SnRK2 proteins were predicted. Physico-chemical characterization revealed these proteins in sizes ranging 53 to 112 kDa with pI values of 4.99 to 9.59. All identified cowpea SnRK2 proteins are hydrophilic in nature. Analysis of the evolutionary relationship of SnRK2 with other related families showed three clusters based on the relatedness to Arabidopsis thaliana and thirteen other crops. Findings of this study provide valuable insights into cowpea SnRK2 gene family and its possible implications in plant stress tolerance.

Keywords

cowpea; genome wide; plant stress; SnRK2; Vigna unquiculata

Introduction

Cowpea (Vigna unguiculata), native from Africa, is widely cultivated in tropical and subtropical regions (1). It is diploid member of the Fabaceae family with a chromosome number 2n = 22 and an estimated genome size of 613 Mb (2).Cowpea has gained more attention recently from consumers and researchers worldwide due to its various health beneficial, including anti-diabetic, anticancer, anti-hyperlipidemic, anti-inflammatory and antihypertensive properties (3). Wide adaptability of cowpea to low fertile soils, pH, high temperatures and drought makes this crop choicest for facing the changed climate scenario. Plants

responses to drought are complex and envisaged with different mechanisms to adapt and survive under drought stresses (4). Drought stressed plants various morphological, physiological, biochemical and molecular changes that adversely affect their growth, development and productivity (1).

Among these tolerance mechanisms, phosphorylation and dephosphorylation are key events modulated by protein families. In plants, major protein kinase groups include calcium-dependent protein kinases, mitogen-activated protein kinases and sucrose non-fermenting-related protein kinases (5). Abiotic stresses trigger a complex osmotic-stress and abscisic acid (ABA) signal transduction network. SNF1related protein kinase2s (SnRK2s) are core ABA signaling components that are activated by ABA-triggered inhibition of type- 2C protein-phosphatases (6). Plant-specific SnRK2 genes play crucial roles in the coordination of plant growth and development and responses to stress and are dubbed as equivalent of mammalian AMP-activated protein kinases and SNF-1 proteins 1 from yeast. SnRK (or SNF1-related protein kinase) family are specific types of serine/threonine protein kinases that exist widely in plants and function significantly in a host of processes, including growth and development, defense against various stresses and hormone-mediated signaling (7, 8). In higher plants, the SnRK family consists of three subfamilies viz., SnRKl, SnRK2 and SnRK3 based on their sequence similarity and C-terminal domain structure characteristics (5). The SnRK2 kinases phosphorylate and thus regulate the activity of downstream components including transcription factors and ion channels (6). Interaction of SnRK2 and protein phosphatase 2C (PP2C) in ABA-dependent signal transduction causes activation of stress-responsive genes (5).

Genetic approaches have played a fundamental role in exploring key genes involved in osmotic stress and ABA signaling pathways. SnRK2 family members have been found in many plant species like Arabidopsis, maize, cotton, rice, wheat, soybean, Rapeseed, crabapple and grapevine (5). However, the SnRK2 family remains unexplored in cowpea. Therefore, the present study aims to identify and characterize the SnRK2 gene family in cowpea using bioinformatics tools.

Materials and Methods

Collection of datasets

The whole cowpea (*Vigna unguiculata*) proteome (assembly ASM411807v1) was downloaded from NCBI (https:// www.ncbi.nlm.nih.gov/genome/). HMMER and keyword searches were performed in the Phytozome V12.1 database (https://phytozome.jgi.doe.gov/pz/portal.html) using the keywords SNF and SnRK2 in the "find genes by keyword" input tab. For reference search using heterologous sequences, *Arabidopsis thaliana* SnRK2 (AtSnRK2) protein sequences were obtained from The Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/index.jsp).

Chromosomal Mapping of SnRK2 genes

Mapgene2chrom, a *tool* to draw gene physical map based on PERL and SVG languages. (http://mg2c.iask.in/ mg2c_v2.0/) was used for chromosome mapping of cowpea SnRK2 genes. The obtained *SnRK2* sequences were mapped on the *Vigna unguiculata* chromosomes using MapGene2Chrom (9) with chromosome co-ordinates (gene id, gene start position, end position, chromosome id and size of chromosome).

Identification of sequence homologs using Hidden Markov models

The HMMER program version 3.3 (http://hmmer.org/), using hidden Markov models, was run to identify *SnRK2* sequences in the cowpea genome by using *AtSnRK2* sequences as bait sequence.

Identification of domain architecture in SnRK2 proteins

The domain and domain architecture of AtSnRK2s were obtained by Simple Modular Architecture Research Tool (SMART) (http://smart.embl-heidelberg.de).

Multiple Sequence Alignment

ClustalW, like other Clustal tools, is used for aligning multiple nucleotide or protein sequences in an efficient manner. Multiple sequence alignments (MSAs) of *AtSnRK2* genes were built using Clustal Omega to validate the results obtained. MSAs were built in MEGA X to confirm the presence of the conserved SnRK2 domain in identified cowpea SnRK2 proteins

Phylogenetic analysis

The evolutionary relationships of cowpea SnRK2 proteins were predicted by building a phylogenetic tree. SnRK2 protein sequences from fifteen different crops were aligned using ClustalW and the tree was constructed by MEGA X (https://www.megasoftware.net/) the neighbour joining method with 1000 bootstrap replications. Further analysis of the phylogenetic tree was performed by exporting this tree to MEGA X and was run by choosing default parameters such as gap penalty and gap extensions with default values

Physico-Chemical analysis of SnRK2 proteins

The theoretical isoelectric point and molecular weight of SnRK2 proteins were determined using ExPASy pI/Mw tool (https://web.expasy.org/compute_pi/).

Results

Whole genome characters of cowpea in NCBI

The results show the chromosome number, reference sequence ID, size of genome sequences, GC content, number of proteins, RNA and gene encoded (Table 1). Gene prediction showed 29,773 total loci containing 42,287 protein-coding transcripts and 12,514 total alternatively spliced transcripts in cowpea.

Characteristics feature of SNF protein in the cowpea genome

The SNF related proteins were characterized and found to be identical across the Cowpea genome. There are 12 protein identities of SnRK2 observed (Table 2).

Chromosomal Mapping: The identified SnRK2 genes were mapped on six chromosomes (chromosomes 1,3,4,8,9 and 11). Four genes, namely 114192996 and 114181284, were predicted

|--|

Туре	Name	RefSeq	INSDC	Size (Mb)	GC%	Protein	Rrna	tRNA	Other RNA	Gene	Pseudogene
Chr	1	NC_040279.1	CM014066.1	42.13	32.8	3,567	-	79	489	2,849	120
Chr	2	NC_040280.1	CM014067.1	33.91	33.3	2,987	10	73	390	2,299	40
Chr	3	NC_040281.1	CM014068.1	65.29	32.0	6,200	-	146	777	4,804	112
Chr	4	NC_040282.1	CM014069.1	42.73	32.9	2,869	3	77	589	2,424	121
Chr	5	NC_040283.1	CM014070.1	48.75	32.7	4,192	-	78	754	3,285	100
Chr	6	NC_040284.1	CM014071.1	34.46	32.8	3,282	-	81	565	2,694	107
Chr	7	NC_040285.1	CM014072.1	40.88	32.0	4,243	-	105	490	3,182	72
Chr	8	NC_040286.1	CM014073.1	38.36	32.9	3,187	-	79	357	2,563	83
Chr 9 NC_040287.1 CM014074.1 43.93 32.7 4,019 32 84 516 3,072 (68			
Chr	10	NC_040288.1	CM014075.1	41.33	33.2	2,888	282	92	542	2,696	113
Chr	11	NC_040289.1	CM014076.1	41.68	33.2	3,079	123	109	538	2,694	130
	Pltd	NC_018051.1	-	0.15	35.2	84	8	38	-	131	1
Un	-	±	-	45.46	36.6	576	828	94	236	1,690	147
Table 2. SNF related protein families in cowpea											

Name	Accession	Start	Stop	Std	Gene ID	Locus	Protein product	Length
VuSnRK2.1	NC_040279.1	18482681	18484506	+	114192996	LOC114192996	XP_027938484.1	471
VuSnRK2.2	NC_040279.1	27455241	27459193	-	114181284	LOC114181284	XP_027923498.1	832
VuSnRK2.3	NC_040289.1	37980286	37985690	+	114168947	LOC114168947	XP_027909735.1	1036
VuSnRK2.4	NC_040281.1	50643408	50646050	+	114178934	LOC114178934	XP_027920886.1	511
VuSnRK2.5	NC_040281.1	61287418	61298513	+	114179887	LOC114179887	XP_027922179.1	703
VuSnRK2.6	NC_040282.1	11541389	11543515	-	114181894	LOC114181894	XP_027924343.1	547
VuSnRK2.7	NC_040286.1	13343970	13349236	-	114194176	LOC114194176	XP_027940068.1	777
VuSnRK2.8	NC_040287.1	32398570	32403138	+	114164083	LOC114164083	XP_027904391.1	537
VuSnRK2.9	NC_040287.1	33057516	33064380	-	114162893	LOC114162893	XP_027902685.1	785
VuSnRK2.10	NC_040287.1	42775720	42778372	-	114164364	LOC114164364	XP_027904808.1	499
VuSnRK2.11	NC_040287.1	17565838	17567763	+	114162141	LOC114162141	XP_027901727.1	528
VuSnRK2.12	NC_040287.1	42901634	42905741	+	114163745	LOC114163745	XP_027903839.1	510

to be located on chromosome 7, whereas 114178934 and 114179887 had predicted locations on chromosomes 3 and 114181894, 114168947 and 114194176 are located chromosome 4, 8 and 11, respectively. 114162141,114162893, 114163745 and 114164364 are located onchromosome 9 (Fig.1).

Identification of SnRK2 proteins in the *Arabidopsis thaliana* database (TAIR) using gene name, GenBank accession and description about SnRK2 protein matched with 17 proteins belonging to SnRK2 protein family.

Sequence homologs and domain architecture of cowpea SnRK2 genes

By using the HMMER program, the domain architectures of cowpea SnRK2s were studied. Search predicted the protein kinase domain belonging to PF00069 super family. SMART detects domains from sequences with relatively high selectivity and specificity. The *Vigna unguiculata* protein SnRK2 is predicted by SMART to contain the



Fig. 1. Chromosomal mapping of cowpea SnRK2 genes.

SnRK2 proteins	Position	E-value	Sequence
VuSnRK2.1	84 to 232	1.00e-20	KKPRQNEKQLPEKASAILPQSALYTQLLDVEARVDAALTRKKVDIQEAMRNPPCIQKTLRIFV FNTFANQSGDSSAPTWTLKIVGRILEDGEEAEQPGVLAHRMPPLYPKFSAFFKRVTISLDKRL YPDNNVITWENSRSSATHEGFE
VuSnRK2.2	361 to 409	1.995e-13	
VuSnRK2.3	120 to 273	3.00e-22	VYNTFSKQVKVEPGKIDVEELSWVLRITGRVLEDGKDSVADGVLSKENRRFSAFFKKITVYLDQ GFYPDNHVVVWDSARSTAQRDGFE
VuSnRK2.4	152 to 308	1.00e-21	SRRKKQKLPEKQLQDKVAAILPESALYTQLLEFESRVDAALARKKADIQEALKNPPCIQKTLRI YVFNTFANQIRTIPKKPTAEPPTWTLKIVGRILEDGVDPDQPGVVQKSTPLYPKFSAFFKRVTI SLDQRLYPDNHIIMWENARSPAPHEGFE
VuSnRK2.5	152 to 308	1.00e-21	SRRKKQKLPEKQLQDKVAAILPESALYTQLLEFESRVDAALARKKADIQEALKNPPCIQKTLRI YVFNTFANQIRTIPKKPTAEPPTWTLKIVGRILEDGVDPDQPGVVQKSTPLYPKFSAFFKRVTI SLDQRLYPDNHIIMWENARSPAPHEGFE
VuSnRK2.6	324 to 368	6.00e-19	LSENYCHYCSRSLPVVYYQSQKEVDILLCTDCFHDGRFVAGHSS
VuSnRK2.7	86 to 220	1.00e-13	LPESALYTQLLDFEAQVDTALARRKFDIQEARLPPHVQKTLRVVVFNTFSNHAKMDSENKKA NESSWSLRITGRILEDGMDSMSGISQRSSPSYPKFSAFFKKITIHLDQSIYPDNHVIVWDSARS PAQQDGFE
VuSnRK2.8	156 to 290	2.00e-13	LPESALYTQLLDFEAQVDTALARRKFDIQEARLPPHVQKTLRVYVFNTFSNHAKMDSENKKA NESSWSLRITGRILEDGMDSMSGISQRSSPSYPKFSAFFKKITIHLDQSIYPDNHVIVWDSARS PAQQDGFE
VuSnRK2.9	192 to 229	2.00e-07	NCGLCGGKYSSGHYRCTQDNFIICANCFKSGNYGEKR
VuSnRK2.10	334 to 378	1.00e-21	LSDNHCIYCSRPLPVVYYQSQKEVDILLCTDCFHDGRFVIGHSS
VuSnRK2.11	238 to 286	4.15294613826537e-11	TKTDWSEKETTNLLEALTHYGDDWKRVSQHVAGRTEKECVAHFLKLPFA
VuSnRK2.12	171 to 219	0.029e-12	SGGKWTDQETLLLHEALELYKENWNEISEHVAAKSKSSITLFQMPIEDA

domain sequence as depicted in Table 3.

Multiple Sequence Alignments: Multiple Sequence Alignments were performed using Clustal Omega to validate the conservation of VuSnRK2 protein sequences and presented (Fig. 2).

Phylogenetic Analysis: The evolutionary relationship of cowpea SnRK2 proteins was predicted by building a phylogenetic tree. cowpea SnRK2 and AtSnRK2 protein sequences were aligned by Mega X and tree was built by the neighbor joining method with 1000 bootstrap replications (Fig. 3). The phylogenetic relationship was done among the 12*VuSnRK2* and 14 different crops SnRK2 protein were determined by constructing a phylogenetic tree by using complete protein sequences of AtSnRK2 and cowpea SnRK2. Based on similarity with AtSnRK2, the identified cowpea SnRK2 were placed into four different groups (I, II, III and IV).

Physico-Chemical Analysis: The theoretical isoelectric point and molecular weight of VuSnRK2 proteins were determined by ExPASy pl/Mw tool. The isoelectric point of proteins ranged from 4.99 to 9.59 and the molecular mass ranged from

XP 027940068.1		181
XP 027902685.1	TKVLVVPMHSDWESPATVHRLER	191
XP 027909735.1		160
XP 027903839.1		0
XP 027904391.1	I PDSDPEI FLYTTPSSSRWFAWDETHETER	43
XP 027904808.1	DPKSSADANVTVVPSYSRWFSWDSTHESEV	81
XP 027901727 1	MPGAROOKKOSPGKGAM-ELPESALYTOLLDEEAOVDTALARRK	181
XP 027901728.1	MPGAROOKKOSPGKGAM-ELPESALYTOLLDEEA0VDTALARRK	181
XP 027920886.1	SAFKTMELTVAPPRKKRSEPEKLTPDKVAK - LVPESATVAKLLELETHTDSVLVRKK	165
XP 027920887.1	SAFKIMEL TVAPPRKKRSEPEKLI TPDKVAK - LVPESATVAKLI ELETHTDSVLVRKK	165
XP 027938484.1	TPLKOTEPVI GPRRKKPRONEKOL PEKASA-TI POSALYTOLI DVEARVDAALTRKK	126
XP 027938490.1	TPL KOTEPVL GPRRKKPRONEKOL PEKASA-TL POSAL YTOL L DVEARVDAAL TRKK	126
XP 027924343.1	SPL RPMEL TPASRRKKOKL PEKOLODKVAA - TL PESALYTOL LEFESRVDAALARKK	197
XP 027924345 1	SPI RPMELTPASRRKKOKI PEKOLODKVAA-TI PESALVTOLI EEESRVDAALARKK	197
XP 027923498.1	LVNSNLADKNRATDTLEPPKSTTRAELLPHOKEGLAWLVRRENTD	219
NP 201489 1		36
XP 027922179.1	DPLVORATAAASAVPDLODRYCKTPSYTESKLLPFOREGVRFTLOHG	211
XP 027940068.1	0AVPHFFSGKSPDHTPEKYMECRNYIVARYMEDPGKRITVPSCOVLSVGVGNEDLTRIVR	241
XP 027902685.1	OAVPHFFSGKSPDHTPEKYMECRNCIVAMHLEOPGKRITLSDCOGLLTGIDVEDLTRIVR	251
XP 027909735.1	OMLPSFFNGKTENRTPDVYMEIRNWIMKKFHSNPNVOIEMKDMSOLNVGD-MDAROEVME	219
XP 027903839.1	MEIRKYLIKRFHTNRNVOFELKDLLEINLGD-VDAROEV/W	40
XP 027904391.1	TAFKEFFDASSISRTPKIYKEYRDFIINKYREEPSRRLTFTEVRKSLVGD-VTFLHKAFL	102
XP 027904808.1	RHLPEFFESASKSPRVYKYYRNSIVKFFRYNPTRKITFTDVRKTLVGD-VGSIRRVFD	138
XP 027901727.1	A-RLPPHVOK	195
XP_027901728.1	A-RLPPHVOK	195
XP_027920886.1	NLRNPRCVRR	180
XP_027920887.1	NLRNPRCVRR	180
XP_027938484.1	IQK	141
XP 027938490.1	VDIOE AMRNPPC IOK	141
XP 027924343.1	ADIOEADIOEALKNPPCIOK	212
XP_027924345.1	ADIQEALKNPPCIQK	212
XP_027923498.1	-DLPPFWEENHSKFVNILTDYQTDKRPDPLRGGIVADEMGLGK	261
NP_201489.1	RLMRDKLTK	45
XP_027922179.1	GRVLLADEMGLGK	224

Fig. 2. Multiple Sequence Alignment results of VuSnRK2 proteins.

53259.04 to 112344.7 Da.

Discussion

Plants cope with changing climatic conditions and evolve self defense mechanism against various stresses. These mechanisms trigger expression of key genes that regulate important metabolic responses (10). Among these inducers, the plant hormone ABA regulates genes involved in growth and development as well as stress responsiveness (11-13). *SnRK2*, a plant-specific serine/threonine kinase has been reported to activate genes in response to biotic and abiotic stress signals (14, 15). Although these genes have been reported in several crop species, only a few have been validated.

Comprehensive analysis of such genes in Vigna family is limited thus demands attention to explore the family in cowpea. In this study, 16 *VuSnRK2* genes were identified from the genome database and designated as*VuSnRK2.1-VuSnRK2.12* (Table 2). Chromosome distribution and physicochemical property analysis revealed that these*VuSnRK2* genes were unevenly distributed. Gene family expansion could be attributed to tandem duplication, segmental duplication and whole genome duplication which drives genetic variation and

XP 027940068.1	FLDOWGIINYCAENPRREYLDNDTCLTEDTSGALC	276
XP 027902685.1	FLDH//GIINYCVOTSSFKSPNVISCLREEPSGEVR	286
XP 027909735.1	FLDYWGLINFHPFPSMD-SAVATASDDGEAEKSSLLEKLYHFETLOLC	266
XP_027903839.1	FLDYWGLINFDSLSSMG-YVDS	61
XP_027904391.1	FLONWGLINYGAPSAADAEKEEEEEEPCKVRLEEGTPNGIR	143
XP_027904808.1	FLEAWGLINVHPSSSLTKPLKWDDKETKS	167
XP_027901727.1	TLRVYVFNTFSNHAKMD-SENKKANESSWSLRITGRILEDGMD-SMSGISQRSSPSYP	251
XP_027901728.1	TLRVYVFNTFSNHAKMD-SENKKANESSWSLRITGRILEDGMD-SMSGISQRSSPSYP	251
XP_027920886.1	TLRIYVYNTFSKQVKVE-PGKIDVEELSWVLRITGRVLEDGKD-SVADGVLSKENR	234
XP_027920887.1	TLRIYVYNTFSKQVKVE-PGKIDVEELSWVLRITGRVLEDGKD-SVADGVLSKENR	234
XP_027938484.1	TLRIFVFNTFANQSGDSSAPTWTLKIVGRILEDGEE-AEQPGVLAHRMPPLYP	193
XP_027938490.1	TLRIFVFNTFANQSGDSSAPTWTLKIVGRILEDGEE-AEQPGVLAHRMPPLYP	193
XP_027924343.1	TLRIYVFNTFANQIRTI-PKKPTAEPPTWTLKIVGRILEDGVD-PDQPGVVQK-STPLYP	269
XP_027924345.1	TLRIYVFNTFANQIRTI-PKKPTAEPPTWTLKIVGRILEDGVD-PDQPGVVQK-STPLYP	269
XP_027923498.1	TLIMLSLIAFDKSQMGV-SRN-GRTDRKWVSVGKRRRNEVEGSHGTGVRTN-GTLVVC	316
NP_201489.1	ELVAVKYIERGDKIDENVQREIIN	69
XP_027922179.1	TLQAIAVASCIVLIIA	245
XP_027940068.1	VPSVALRSIDSLIEFDKPKCKFKADEVYSCRTMHNTDISDLDDT	320
XP_027902685.1	VPVETLKSIDSLIKFDNPSCKLKADEIYSSLTAHNVDVFDLEDR	330
XP_027909735.1	RPVQRSSQMTPATASGLFPESTIAEE-LVKQ-EG	298
XP_027903839.1	VKQ-EE	66
XP_027904391.1	V-AATPNSLKPMLVPRGAKTGGNATAASLKLPPLASYSDIYG	184
XP_027904808.1	DSASNSTEPSSAP	180
XP_027901727.1	KFSAFFKKITIHLDQSIYPDNHVIWDSARSPAQQDGFEVKRK-GNKEFTALIAMEMNYT	310
XP_027901728.1	KFSAFFKKITIHLDQSIYPDNHVIWDSARSPAQQDGFEVKRK-GNKEFTALIAMEMNYT	310
XP_027920886.1	RFSAFFKKITVYLDQGFYPDNHVVWDSARSTAQRDGFEVKRK-GDKEFTAVVRMSMNYS	293
XP_027920887.1	RFSAFFKKITVYLDQGFYPDNHVVWDSARSTAQRDGFEVKRK-GDKEFTAVVRMSMNYS	293
XP_027938484.1	KFSAFFKRVTISLDKRLYPDNNVITWENSRSSATHEGFEVKRK-GDKEFSAQIRLEMNYV	252
XP_027938490.1	KFSAFFKRVTISLDKRLYPDNNVITWENSRSSATHEGFEVKRK-GDKEFSAQIRLEMNYV	252
XP_027924343.1	KFSAFFKRVTISLDQRLYPDNHIIMWENARSPAPHEGFEVKRK-GDKEFAVNIRLEMNYV	328
XP_027924345.1	KFSAFFKRVTISLDQRLYPDNHIIMWENARSPAPHEGFEVKRK-GDKEFAVNIRLEMNYV	328
XP_027923498.1	PPSVMSAWITQLEDHTVHGGLKTYMYYGEKRTKDIEE	353
NP_201489.1	HRSLRHPNIVRFKEVILTPTHLAIIMEYASGGELYERICNAGRFSEDEARFFFQQLLS	127
XP_027922179.1	PSSLRLQWASMIQQWLNIPSSDILIVLPQSGGSNRGGFNIVSSSSKNSIRLNGLFN	301
C 2 C 2 C 2 C 2 C 2 C 2 C 2 C 2 C 2 C 2		



Fig. 3. Evolutionary relationship of cowpea SnRK2 proteins- Phylogenetic tree constructed using MEGA X tool.

adaptation under natural selection (16, 17).

The evolutionary relationship between *VrSnRK2* and *AtSnRK2* was examined through a comprehensive phylogenetic analysis performed by constructing a phylogenetic tree. The result showed that *VrSnRK2* genes were clustered into four groups (I, II, III and IV) (18, 19).

The Group I comprised of *Zea mays, Oryza sativa* (Japonica group), *Hordeum vulgare, Solanum tuberosum, Arabidopsis thaliana, Malus prunifolia, Litchi chinensis* and *Manihot esculenta.* Group II included *Trifolium pratense, Solanum chacoense, Heaveabrasilensis, Vigna unguiculata* (VuSnRK 2.1, 2.8, 2.9 and 2.12). Group III consisted of *Arabidopsis thaliana* 2.3, VuSnRK2.2,2.6 and 2.7, *Nicotiana plumbaginifolia, Triticum aestivum, Medicago sativa, Populus tremuloides, Camellia sinensis, Populus tremula, Gossypium hirsutum* whereas Group IV included *Vigna unguiculata* (VuSnRK 2.3, 2.4, 2.5, 2.10 and 2.11).

These groupings align with previously reported classifications as in Arabidopsis (20), wheat (21), cotton (19), sugarcane (*Saccharum officinarum* L.) and maize (22), where*SnRK2* genes have been categorized. These results suggest that *VuSnRK2* might have been evolved from a common ancestor and has been later diverged after the segregation of monocots and dicots.

Furthermore, gene descriptions obtained from the TAIR database search has shown the possible role of these genes in abiotic stress signaling. These findings corroborates with previous reports and adds value of information that SnRK2s are indeed involved in signaling pathways related to drought-stress (23, 24). Future research of these identified proteins could demonstrate the possible role of VuSnRK2 proteins in the stress responses.

Conclusion

In the present study, twelve*SnRK2* genes in *Vigna unguiculata* were identified and *in silico* analysis has determined the gene structure, transcript length, chromosomal location and evolutionary relationships of this *SnRK2* gene family in *Vigna unguiculata* with other crop species with similar functions. These findings shed light on the possible role of *SnRK2* genes in *Vigna unguiculata* and provide a foundation for further functional validation studies.

Acknowledgements

We gratefully acknowledge the crews of respective bioinformatics online platforms for making the computational biology data/tools publicly available. We express our sincere gratitude to the Director, Centre for Plant Molecular Biology and Biotechnology for the provision of data analysis facilities.

Authors' contributions

The manuscript was developed through a collaborative effort among the authors. RS and RSV conceptualized the research, wrote the original draft. RS involved in overall supervision of the work. KS involved in use of methodology, data curation and formal analysis using softwares. NS, RS, RT and AT involved in review, editing and proof reading. All authors read and approved the final version of the manuscript, ensured its readiness for submission.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

- Carvalho M, Castro I, Moutinho-Pereira J, Correia C, Egea-Cortines M, Matos M, et al. Evaluating stress responses in cowpea under drought stress. J Plant Physiol. 2019;241:153001. https:// doi.org/10.1016/j.jplph.2019.153001
- Lonardi S, Muñoz-Amatriaín M, Liang Q, Shu S, Wanamaker SI, Lo S, et al. The genome of cowpea (Vigna unguiculata [L.] Walp.). Plant J. 2019;98(5):767-82. https://doi.org/10.1111/tpj.14382
- Jayathilake C, Visvanathan R, Deen A, Bangamuwage R, Jayawardana BC, Nammi S, et al. Cowpea: an overview on its nutritional facts and health benefits. J Sci Food Agric. 2018;98 (13):4793-806. https://doi.org/10.1002/jsfa.9074
- Carvalho M, Lino-Neto T, Rosa E, Carnide V. Cowpea: a legume crop for a challenging environment. J Sci Food Agric. 2017;97 (13):4273-84. https://doi.org/10.1002/jsfa.8250
- Fatima A, Khan MJ, Awan HM, Akhtar MN, Bibi N, Sughra K, et al. Genome-wide identification and expression analysis of SnRK2 gene family in mungbean (Vigna radiata) in response to drought stress. Crop Pasture Sci. 2020;71(5):469-76. https:// doi.org/10.1071/CP19392
- Takahashi Y, Zhang J, Hsu PK, Ceciliato PH, Zhang L, Dubeaux G, et al. MAP3Kinase-dependent SnRK2-kinase activation is required for abscisic acid signal transduction and rapid osmotic stress response. Nat Commun. 2020;11(1):12. https://doi.org/10.1038/ s41467-019-13875-y
- Wu Z, Cheng J, Hu F, Qin C, Xu X, Hu K. The SnRK2 family in pepper (Capsicum annuum L.): Genome-wide identification and expression analyses during fruit development and under abiotic stress. Genes & Genomics. 2020;42:1117-30. https:// doi.org/10.1007/s13258-020-00968-y
- Ahmed B, Hasan F, Tabassum A, Ahmed R, Hassan R, Amin MR, et al. Genome-wide investigation of SnRK2 gene family in two jute species: Corchorus olitorius and Corchorus capsularis. J Genet Eng Biotechnol. 2023;21(1):5. https://doi.org/10.1186/s43141-022-00453-x
- Chao JT, Kong YZ, Wang Q, Sun YH, Gong DP, Lv J, et al. MapGene2Chrom, a tool to draw gene physical map based on Perl and SVG languages. Yi chuan. 2015;37(1):91-7. https:// doi.org/10.16288/j.yczz.2015.01.013
- 10. Bohnert HJ, Gong Q, Li P, Ma S. Unraveling abiotic stress tolerance mechanisms–getting genomics going. Curr Opin Plant Biol. 2006;9(2):180-88. https://doi.org/10.1016/j.pbi.2006.01.003
- Golldack D, Li C, Mohan H, Probst N. Gibberellins and abscisic acid signal crosstalk: living and developing under unfavorable conditions. Plant Cell Rep. 2013;32:1007-16. https://doi.org/10.1007/s00299-013-1409-2

- Verma V, Ravindran P, Kumar PP. Plant hormone-mediated regulation of stress responses. BMC plant biology. 2016;16:1-10. https://doi.org/10.1186/s12870-016-0771-y
- Hasan MM, Liu XD, Waseem M, Guang-Qian Y, Alabdallah NM, Jahan MS, et al. ABA activated SnRK2 kinases: An emerging role in plant growth and physiology. Plant Signal Behav. 2022;17 (1):2071024. https://doi.org/10.1080/15592324.2022.2071024
- Coello P, Hey SJ, Halford NG. The sucrose non-fermenting-1related (SnRK) family of protein kinases: potential for manipulation to improve stress tolerance and increase yield. J Exp Bot. 2011;62(3):883-93.https://doi.org/10.1093/jxb/erq331
- Liu J, Wei L, Wu Y, Wang Z, Wang H, Xiao J, et al. Characterization of sucrose nonfermenting-1-related protein kinase 2 (SnRK2) gene family in Haynaldiavillosa demonstrated SnRK2. 9-V enhances drought and salt stress tolerance of common wheat. BMC Genomics. 2024;25(1):209. https://doi.org/10.1186/s12864-024-10114-7
- Cronk QC. Plant evolution and development in a post-genomic context. Nat Rev Genet. 2001;2(8):607-19. https:// doi.org/10.1038/35084556
- Wei K, Wang Y, Xie D. Identification and expression profile analysis of the protein kinase gene superfamily in maize development. Mol Breed 2014;33:155-72. https://doi.org/10.1007/s11032-013-9941-x
- Umezawa T, Nakashima K, Miyakawa T, Kuromori T, Tanokura M, Shinozaki K, et al. Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. Plant Cell Physiol. 2010;51(11):1821-39. https://doi.org/10.1093/pcp/pcq156
- Liu Z, Ge X, Yang Z, Zhang C, Zhao G, Chen E, et al. Genome-wide identification and characterization of SnRK2 gene family in cotton (Gossypium hirsutum L.). BMC Genet. 2017;18:1-4. https:// doi.org/10.1186/s12863-017-0517-3
- Hrabak EM, Chan CW, Gribskov M, Harper JF, Choi JH, Halford N, et al. The Arabidopsis CDPK-SnRK superfamily of protein kinases. Plant Physiol. 2003;132(2):666-80. https://doi.org/10.1104/ pp.102.011999
- Zhang H, Li W, Mao X, Jing R, Jia H. Differential activation of the wheat SnRK2 family by abiotic stresses. Front Plant Sci. 2016;7:420. https://doi.org/10.3389/fpls.2016.00420
- Huai J, Wang M, He J, Zheng J, Dong Z, Lv H, et al. Cloning and characterization of the SnRK2 gene family from Zea mays. Plant Cell Rep. 2008;27:1861-68. https://doi.org/10.1007/s00299-008-0608-8
- Lou D, Wang H, Liang G, Yu D. OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice. Front Plant Sci. 2017;8:993. https://doi.org/10.3389/fpls.2017.00993
- Wang X, Wang Y, Wang L, Liu H, Zhang B, Cao Q, et al. Arabidopsis PCaP2 functions as a linker between ABA and SA signals in plant water deficit tolerance. Front Plant Sci. 2018;9:578. https:// doi.org/10.3389/fpls.2018.00578