Stallion Journal for Multidisciplinary Associated Research Studies
ISSN (Online): 2583-3340
Volume-3 Issue-1 || February 2024 || PP. 37-42 https://doi.org/10.55544/sjmars.3.1.5

In Silico Analysis of CKIN/SnRK Genes for Osmotic Stress Responsive in Genomic Elements Pigeon Pea (*Cajanus cajan* L.)

Arti[1], Shobhit Kumar Srivastava[2] and Manoj Kumar Sharma[3]
[1]Department of Botany, Asian International University, Imphal West, Manipur, INDIA.
[2]Associate Professor, Department of Botany, Asian International University, Imphal West, Manipur, INDIA.
[3]Department of Bioinformatics, Janta Vedic College, Baraut, Baghpat, Uttar Pradesh, INDIA.

www.sjmars.com || Vol. 3 No. 1 (2024): February Issue

Date of Submission: 15-03-2024	**Date of Acceptance:** 21-03-2024	**Date of Publication:** 26-03-2024

ABSTRACT

The SnRK2 (SNF1-related protein kinase 2) gene family is a critical regulator of abscisic acid (ABA) signaling pathways, orchestrating plant responses to abiotic stresses such as drought and salinity. In the pigeon pea (*Cajanus cajan* L.), a diploid legume adapted to semi-arid tropics, an extraordinary expansion of this gene family has been identified, with 1,210 putative genes. This study focuses on 245 CcSnRK2 genes possessing the Pkinase_Tyr domain, exploring their structural diversity, functional domains, subcellular localization, conserved motifs, physicochemical properties, and phylogenetic relationships. Homology-based identification using BLASTP and Conserved Domain Database (CDD) analysis confirmed the presence of the Pkinase_Tyr domain, suggesting potential dual-specificity kinase activity. Notably, 46 genes exhibit nuclear localization, indicating roles in transcriptional regulation of stress-responsive genes. Phylogenetic analysis, conducted using ClustalW and MEGA 6.0, grouped these genes into four clades, reflecting functional diversification likely driven by gene duplication and polyploidy. Physicochemical properties, analyzed *via* ProtParam, reveal diverse protein architectures suited for stress adaptation. These findings highlight the SnRK2 family's pivotal role in pigeon pea's resilience and provide a foundation for molecular breeding to enhance crop tolerance in challenging environments.

Keywords- SnRK2 gene, ABA, Pigeon pea, Pkinase_Tyr domain.

I. INTRODUCTION

Pigeon pea (*Cajanus cajan* L.) is a vital legume crop cultivated in semi-arid tropics, prized for its protein-rich seeds and remarkable resilience to abiotic stresses such as drought and salinity (Varshney *et al*., 2012). As a diploid species (2n=2x=22, genome size ~833 Mb), pigeon pea thrives in resource-poor environments, making it a cornerstone of food security in regions prone to environmental challenges. Central to its stress tolerance is the SnRK2 (SNF1-related protein kinase 2) gene family, a group of serine/threonine/tyrosine protein kinases that mediate abscisic acid (ABA) signaling, a key pathway regulating plant responses to abiotic stresses (Kulik *et al*., 2011). SnRK2 genes are critical for processes such as stomatal closure, seed dormancy, and stress-responsive gene expression, enabling plants to adapt to adverse conditions (Fujii *et al*., 2009).

In contrast to model plants like *Arabidopsis thaliana* (10 SnRK2 genes) and rice (11 genes), pigeon pea exhibits an unprecedented expansion of the SnRK2 family, with 1,210 putative genes identified (Umezawa *et al*., 2010; Saha *et al*., 2014). This expansion, likely driven by genome duplication events and the tetraploid nature of pigeon pea, suggests a robust genetic framework for stress adaptation (Varshney *et al*., 2012). The presence of the Pkinase_Tyr domain in 245 of these genes indicates potential dual-specificity kinase activity, a rare feature in plants that may enhance signaling complexity by facilitating cross-talk with other pathways, such as MAPK cascades (Rudrabhatla *et al*., 2006; Ghelis *et al*., 2008).

This study comprehensively investigates 245 CcSnRK2 genes, focusing on their identification, functional domains, subcellular localization, conserved motifs, physicochemical properties, and phylogenetic relationships. Using

bioinformatics tools such as TBtools, MEME Suite, ProtParam, ClustalW, and MEGA 6.0, we aim to elucidate the structural and functional diversity of these genes. Particular attention is given to 46 nuclear-localized genes, which may play a central role in transcriptional regulation of stress responses. The findings aim to deepen our understanding of pigeon pea's molecular resilience and provide insights for molecular breeding to develop stress-tolerant cultivars, enhancing agricultural sustainability in semi-arid regions.

II. METHODOLOGY

Gene Identification

Putative CcSnRK2 genes were identified in the pigeon pea genome using homology-based approaches. BLASTP searches were performed against known SnRK2 sequences from *Arabidopsis thaliana*, rice, and other plants, with an e-value threshold of 1e-10 to ensure specificity. Candidate genes were further validated for the presence of the Pkinase_Tyr domain (Pfam00069 or cl38241) using the Conserved Domain Database (CDD) at NCBI (Marchler-Bauer *et al*., 2017). Genomic annotations and visualizations were generated using TBtools, a bioinformatics toolkit for interactive analysis of large biological datasets (Chen *et al*., 2020). This approach ensured accurate identification and annotation of the 1,210 putative CcSnRK2 genes.

Functional Domain Analysis

The 245 CcSnRK2 genes with the Pkinase_Tyr domain were analyzed using CDD to confirm domain presence and assess structural features. TBtools was employed to map domain distributions across the genome and visualize gene organization, providing insights into potential functional roles. The analysis focused on the Pkinase_Tyr domain.

Subcellular Localization Prediction

Subcellular localization of the 245 CcSnRK2 gene products was predicted using computational tools WoLF PSORT and TargetP (Horton *et al*., 2007). These tools generated localization scores for cellular compartments, including the nucleus, cytoplasm, chloroplast, mitochondria, extracellular space, Golgi/plasma membrane, vacuole, peroxisome, and endoplasmic reticulum. A heatmap of nuclear-localized genes was created using TBtools to highlight genes with strong nuclear localization signals, which are likely involved in transcriptional regulation.

Motif Analysis

Conserved motifs within the 46 nuclear-localized CcSnRK2 genes were identified using the MEME Suite, with parameters set to detect three motifs of 6–50 residues (Bailey *et al*., 2009). These motifs were aligned with the Pkinase_Tyr domain to evaluate their functional relevance, particularly in phosphorylation activity. Phylogenetic clustering of these genes was performed using MEGA 6.0, and results were visualized with TBtools to assess structural and regulatory relationships.

Physicochemical Properties

Physicochemical properties of the 46 nuclear-localized CcSnRK2 genes were computed using the ProtParam tool on the ExPASy server (Gasteiger *et al*., 2005). Parameters included amino acid length, molecular weight (Mw), theoretical isoelectric point (pI), instability index, aliphatic index (AI), and grand average of hydropathicity (GRAVY). These properties provided insights into protein size, charge, stability, thermostability, and hydrophobicity, which influence functional roles and localization.

Phylogenetic Analysis

Full-length amino acid sequences of the 46 nuclear-localized CcSnRK2 genes were aligned using ClustalW ver. 2.1, employing a progressive alignment algorithm for precision (Larkin *et al*., 2007). A phylogenetic tree was constructed in MEGA 6.0 using the neighbor-joining method, Poisson substitution model, uniform rates, and pairwise deletion to handle alignment gaps (Tamura *et al*., 2013). The tree's robustness was assessed with 1,000 bootstrap iterations, and visualization was performed using the Interactive Tree of Life (iTOL) tool (Letunic & Bork, 2021).

III. RESULTS AND DISCUSSION

Gene Identification

The identification of 1,210 putative CcSnRK2 genes in the pigeon pea genome represents a remarkable expansion compared to model plants like *Arabidopsis thaliana* (10 genes) and rice (11 genes) (Umezawa *et al*., 2010; Saha *et al*., 2014). This large repertoire is likely a consequence of genome duplication events, possibly linked to pigeon pea's tetraploid ancestry, which has facilitated the retention of duplicated genes (Varshney *et al*., 2012). The structural diversity of these genes is evident in their varying lengths, ranging from 156 bp (CcSnRK2.688) to 6,000 bp (CcSnRK2.1118), and amino acid counts from 51 to 1,999. For example, CcSnRK2.136 (4,758 bp, 585 amino acids) may contain extensive intronic regions or alternative splicing variants, suggesting functional diversification within the family (Zhao *et al*., 2017). This expansion likely enhances pigeon pea's adaptive capacity to abiotic stresses, such as drought and salinity, prevalent in its semi-arid habitat. Comparative analyses with other legumes, such as soybean (66 SnRK2 genes), indicate that such expansions are associated with enhanced stress resilience (Zhao *et al*., 2017). The homology-based identification, validated

by CDD analysis, underscores the reliability of these findings, though experimental validation through expression profiling or gene editing is necessary to confirm their roles.

Functional Domains

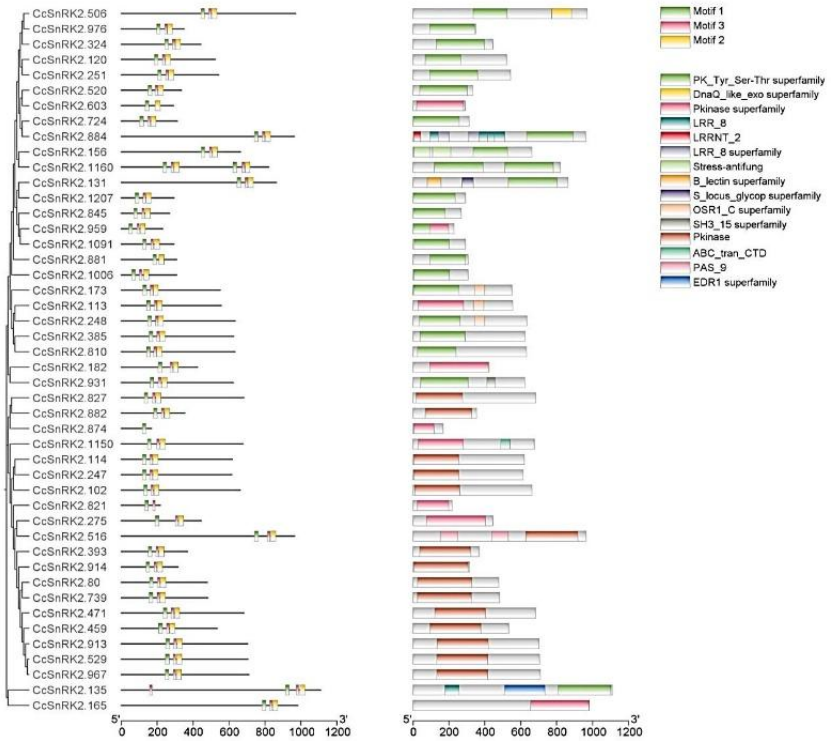
Of the 1,210 CcSnRK2 genes, 245 possess the Pkinase_Tyr domain, a hallmark of potential dual-specificity kinase activity capable of phosphorylating both serine/threonine and tyrosine residues (Rudrabhatla *et al*., 2006). This is particularly significant in plants, where tyrosine phosphorylation is less common but can facilitate cross-talk with other signaling pathways, such as MAPK cascades (Ghelis *et al*., 2008). The large number of CcSnRK2 genes suggests evolutionary pressures to diversify stress response mechanisms, enabling pigeon pea to thrive in challenging environments. The Pkinase_Tyr domain's catalytic properties, including ATP binding and phosphate transfer, are critical for kinase function in ABA signaling, regulating processes like stomatal closure and stress-responsive gene expression (Fujii *et al*., 2009). The use of TBtools for visualizing domain distribution revealed potential clustering patterns, which may indicate coordinated regulation or recent duplication events, as observed in other plant species (Wang *et al*., 2019).

Subcellular Localization

Localization predictions for the 245 CcSnRK2 genes revealed diverse cellular compartments, with 46 genes showing nuclear localization, as indicated by scores ranging from 1 to 13. Notable examples include CcSnRK2.506 (score 13), CcSnRK2.120 (score 12.5), and CcSnRK2.131 (score 12), suggesting strong nuclear presence (Horton *et al*., 2007). These genes are likely involved in phosphorylating nuclear transcription factors, such as AREB/ABF, which mediate stress-responsive gene expression in ABA signaling pathways (Fujii *et al*., 2009; Kulik *et al*., 2011). The nuclear localization aligns with the SnRK2 family's role in transcriptional regulation under stress conditions. The presence of the Pkinase_Tyr domain in these genes suggests potential tyrosine phosphorylation, which could enhance signaling complexity by interacting with other nuclear pathways, such as MAPK cascades (Ghelis *et al*., 2008). The heatmap generated using TBtools highlighted the distribution of nuclear-localized genes, providing a visual representation of their potential regulatory roles.

Motif Analysis

MEME Suite analysis identified three conserved motifs in the 46 nuclear-localized CcSnRK2 genes, likely corresponding to kinase-specific catalytic loops, substrate-binding regions, or ATP-binding domains (Bailey *et al*., 2009). These motifs, aligned with the Pkinase_Tyr domain, suggest functional conservation critical for phosphorylation activity. The presence of these motifs in nuclear-localized genes, such as CcSnRK2.506 and CcSnRK2.120, supports their role in transcriptional regulation, potentially phosphorylating transcription factors under stress conditions (Fujii *et al*., 2009). The potential dual-specificity kinase activity, indicated by the Pkinase_Tyr domain, may enable these genes to modulate diverse substrates, enhancing signaling complexity (Ghelis *et al*., 2008). Phylogenetic clustering using MEGA 6.0 revealed evolutionary relationships among these genes, suggesting that motif conservation is linked to functional diversification, possibly driven by gene duplication events common in legume genomes (Varshney *et al*., 2012).



Stallion Journal for Multidisciplinary Associated Research Studies
ISSN (Online): 2583-3340
Volume-3 Issue-1 || February 2024 || PP. 37-42 https://doi.org/10.55544/sjmars.3.1.5

Physicochemical Properties

ProtParam analysis of the 46 nuclear-localized CcSnRK2 genes revealed a wide range of physicochemical properties, reflecting diverse protein architectures. Molecular weights ranged from 18,396.13 Da (CcSnRK2.874) to 124,413.81 Da (CcSnRK2.135), indicating varied protein sizes that may accommodate multiple phosphorylation sites or regulatory domains (Gasteiger *et al*., 2005). Isoelectric point (pI) values spanned 5.12 (CcSnRK2.156) to 9.57 (CcSnRK2.913), with basic proteins (e.g., CcSnRK2.80, pI 9.19) likely favoring nuclear localization due to their charge properties (Horton *et al*., 2007). Most proteins were stable (instability index <50), except for CcSnRK2.120 (57.01), which may undergo rapid turnover under specific conditions. High aliphatic indices, such as 103.74 for CcSnRK2.884, suggest thermostability, an adaptation to temperature fluctuations in pigeon pea's habitat. Negative GRAVY scores (e.g., -1.265 for CcSnRK2.165) indicate hydrophilicity, facilitating interactions in aqueous cellular environments, which is advantageous for kinase activity (Jain *et al*., 2018). These properties align with the SnRK2 family's role in stress signaling, with diverse architectures supporting specialized functions.

Table: Physicochemical Properties of CcSnRK2 Genes in *Cajanus cajan* L.

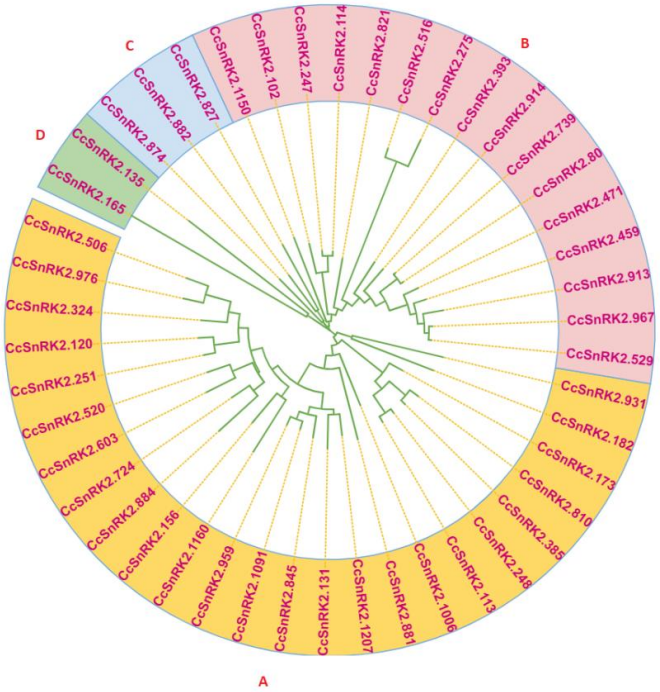
Gene_id	Amino_acid	Molecular_weight	Theoretical_pi	Instability_index	Aliphatic_index	GRAVY
CcSnRK2.80	478	53663.91	9.19	40.47	65.08	-0.778
CcSnRK2.102	662	73059.96	5.33	39.19	39.19	-0.565
CcSnRK2.113	556	64396.39	5.19	48.7	78.54	-0.604
CcSnRK2.114	619	69417.27	9.26	46.89	46.89	-0.493
CcSnRK2.120	523	57392.45	9.18	57.01	65.62	-0.702
CcSnRK2.131	863	95376.01	5.55	31.3	83.92	-0.138
CcSnRK2.135	1110	124413.81	5.8	50.03	86.02	-0.389
CcSnRK2.156	663	73956.13	5.12	44.04	80.18	80.18
CcSnRK2.165	983	114079.1	7.7	50.19	58.13	-1.265
CcSnRK2.173	551	62618.33	5.32	46.4	86.5	-0.38
CcSnRK2.182	425	49581.07	8.75	46.84	82.56	-0.48
CcSnRK2.247	615	68632.59	9.33	48.15	81.35	-0.457
CcSnRK2.248	635	72898.58	5.52	51.29	72.31	-0.636
CcSnRK2.251	543	60208.1	7.18	45.36	70.04	-0.696
CcSnRK2.275	445	49507.64	7.25	54.26	80.29	-0.214
CcSnRK2.324	444	49330.28	8.74	39.44	101.78	-0.077
CcSnRK2.385	625	71375.89	6.42	46.6	74.42	-0.475
CcSnRK2.393	369	42568.64	5.74	40.66	95.37	-0.275
CcSnRK2.459	533	59653.27	9.38	48.47	75.59	-0.559
CcSnRK2.471	683	76042.06	9.24	37.54	68.7	-0.638
CcSnRK2.506	971	108797.64	6.61	48.05	81.93	-0.454
CcSnRK2.516	965	109028.46	7.84	44.83	73.87	-0.661
CcSnRK2.520	334	37232.58	6.04	41.44	90.45	-0.251
CcSnRK2.529	705	78301.56	9.35	53.49	70.5	-0.67
CcSnRK2.603	291	32609.39	7.06	35.49	89.42	-0.281
CcSnRK2.724	312	35035.25	6.04	31.7	91.22	-0.247
CcSnRK2.739	482	54148.51	9.37	37.16	64.75	-0.774
CcSnRK2.810	632	72264.51	5.8	50.62	71.09	-0.492
CcSnRK2.821	217	24578.32	6.44	40.91	90.74	-0.178
CcSnRK2.827	683	76618	6.18	43.07	84.96	-0.434
CcSnRK2.845	269	29845.36	5.57	33.64	95.28	-0.002
CcSnRK2.874	168	18396.13	8.41	38.44	100.95	-0.011
CcSnRK2.881	308	35275.42	6.45	43.84	87.31	-0.425
CcSnRK2.882	354	39791.93	6.08	45.44	95.17	-0.161
CcSnRK2.884	963	106524.41	5.99	35.21	103.74	-0.081
CcSnRK2.913	703	78706.66	9.57	49.12	78.19	-0.629
CcSnRK2.914	316	35824.43	8.91	36.84	94.08	-0.321
CcSnRK2.931	623	69382.92	6.25	42.11	85.23	-0.269
CcSnRK2.959	230	25954.82	6.18	47.49	88.57	-0.235
CcSnRK2.967	710	78463.86	9	49.07	71.9	-0.618
CcSnRK2.976	350	39855.45	7.65	37.84	78.83	-0.542

Stallion Journal for Multidisciplinary Associated Research Studies
ISSN (Online): 2583-3340
Volume-3 Issue-1 || February 2024 || PP. 37-42 https://doi.org/10.55544/sjmars.3.1.5

CcSnRK2.1006	308	34927.3	5.96	49.42	85.84	-0.174
CcSnRK2.1091	292	33006.05	6.46	28.59	88.32	-0.141
CcSnRK2.1150	677	75521.21	7.95	49.2	66.28	-0.666
CcSnRK2.1160	821	92554.78	8.62	43.4	85.81	-0.328
CcSnRK2.1207	292	33645.15	5.59	50.26	75.41	-0.418

Phylogenetic Analysis

The phylogenetic tree of the 46 nuclear-localized CcSnRK2 genes, constructed using ClustalW and MEGA 6.0, was divided into four clades: Clade A (25 genes), Clade B (16 genes), Clade C (3 genes), and Clade D (2 genes) (Tamura *et al*., 2013). Clade A, including genes like CcSnRK2.506 and CcSnRK2.324, exhibited diverse physicochemical properties, such as high aliphatic indices (e.g., 101.78 for CcSnRK2.324), suggesting broad stress adaptation. Clade B, with genes like CcSnRK2.80 (pI 9.19) and CcSnRK2.913 (pI 9.57), likely specializes in nuclear signaling due to high pI values favoring nuclear localization (Horton *et al*., 2007). Clades C and D, with fewer members (e.g., CcSnRK2.874 in Clade C and CcSnRK2.135 in Clade D), may represent specialized lineages arising from recent duplications. The tree's robust methodology, supported by 1,000 bootstrap iterations and visualized using iTOL, indicates high confidence in its branching structure (Letunic & Bork, 2021). The Pkinase_Tyr domain's presence across all clades suggests conserved kinase activity, with potential tyrosine phosphorylation enhancing signaling complexity (Ghelis *et al*., 2008). The tree reflects gene duplication-driven diversification, a common feature in legume genomes, supporting pigeon pea's stress resilience (Varshney *et al*., 2012).



IV. CONCLUSION

The identification of 1,210 putative CcSnRK2 genes in the pigeon pea genome, with 245 possessing the Pkinase_Tyr domain, represents a remarkable genomic expansion, likely driven by polyploidy and segmental duplications (Varshney *et al*., 2012). This expansion underscores pigeon pea's adaptive capacity to thrive in semi-arid environments, where abiotic stresses like drought and salinity are prevalent. The 46 nuclear-localized genes, characterized by conserved motifs, diverse physicochemical properties, and phylogenetic clustering into four clades, highlight their critical role in ABA-mediated stress responses. The presence of the Pkinase_Tyr domain suggests potential dual-specificity kinase activity, enabling phosphorylation of serine/threonine and tyrosine residues, which may facilitate cross-talk with other signaling pathways (Rudrabhatla *et al*., 2006; Ghelis *et al*., 2008). Clade A's diversity and Clade B's nuclear signaling focus indicate broad and specialized stress adaptation, respectively, while smaller Clades C and D suggest niche roles. The physicochemical properties, including thermostability and hydrophilicity, align with the SnRK2 family's function in stress signaling, supporting pigeon pea's resilience (Jain *et al*., 2018). These findings provide a comprehensive framework for understanding the molecular basis of pigeon pea's stress tolerance and offer valuable resources for molecular breeding to develop resilient cultivars, enhancing food security in marginal environments.

FUTURE RECOMMENDATIONS

Future research on CcSnRK2 genes should focus on experimental validation through transcriptomic studies under drought and salinity stress, using RNA sequencing and quantitative PCR to identify key stress-responsive genes in Clades A and B. CRISPR/Cas9 can confirm roles of genes like CcSnRK2.506 and CcSnRK2.120 in transcriptional regulation. Mass spectrometry should validate physicochemical properties, while biochemical assays explore tyrosine phosphorylation in Pkinase_Tyr domains for MAPK pathway interactions. Comparative genomics with legumes and genome mapping of 1,210 CcSnRK2 genes may reveal conserved patterns. These insights can guide molecular breeding for stress-resilient pigeon pea cultivars, enhancing sustainable agriculture and food security.

REFERENCES

[1] Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., ... & Noble, W. S. (2009). MEME Suite: Tools for motif discovery and searching. *Nucleic Acids Research*, 37(Web Server issue), W202-W208

[2] Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., & Xia, R. (2020). TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Molecular Plant*, 13(8), 1194–1202.

[3] Fujii, H., Chinnusamy, V., Rodrigues, A., Rubio, S., Antoni, R., Park, S. Y., ... & Zhu, J. K. (2009). In vitro reconstitution of an abscisic acid signalling pathway. *Nature*, 462(7273), 660-664.

[4] Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. In *The Proteomics Protocols Handbook* (pp. 571-607). Humana Press.

[5] Ghelis, T., Loudet, O., Serra, T. S., Goasdoue, N., & Meyer, C. (2008). Tyrosine phosphorylation in plant signalling. *Plant Signaling & Behavior*, 3(11), 911-913.

[6] Horton, P., Park, K. J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., & Nakai, K. (2007). WoLF PSORT: Protein localization predictor. *Nucleic Acids Research*, 35(Web Server issue), W585-W587.

[7] Jain, P., Shah, K., & Rishi, V. (2018). Tyrosine phosphorylation-mediated signaling in *Cajanus cajan* under abiotic stress. *Plant Physiology and Biochemistry*, 133, 76-85.

[8] Kulik, A., Wawer, I., Krzywińska, E., Bucholc, M., & Dobrowolska, G. (2011). SnRK2 protein kinases—key regulators of plant response to abiotic stresses. *OMICS: A Journal of Integrative Biology, 15*(12), 859–872.

[9] Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., ... & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23(21), 2947-2948.

[10] Letunic, I., & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, 49(W1), W293–W296.

[11] Marchler-Bauer, A., Bo, Y., Han, L., He, J., Lanczycki, C. J., Lu, S., Bryant, S. H. (2017). CDD/SPARCLE: Functional classification of proteins via subfamily domain architectures. *Nucleic Acids Research*, 45(D1), D200–D203.

[12] Rudrabhatla, P., Reddy, M. M., & Rajasekharan, R. (2006). Genome-wide analysis and experimentation of plant serine/threonine/tyrosine-specific protein kinases. *Plant Molecular Biology*, 60(2), 293-319.

[13] Saha, J., Chatterjee, C., Sengupta, A., Gupta, K., & Gupta, B. (2014). Genome-wide analysis and evolutionary study of sucrose non-fermenting 1-related protein kinase 2 (SnRK2) gene family members in Arabidopsis and rice. *Computational Biology and Chemistry*, 49, 59-70.

[14] Satyam, R., Ahmad, S., & Jatav, P. K. (2024). Identification and characterization of nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes in the *Cajanus cajan* genome and their expression profiling in response to salinity stress. *Plant Gene*, 37, 100446.

[15] Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729.

[16] Umezawa, T., Yoshida, R., Maruyama, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2004). SRK2C, a SNF1-related protein kinase 2, improves drought tolerance by controlling stress-responsive gene expression in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences, 101*(49), 17306–17311.

[17] Varshney, R. K., Chen, W., Li, Y., Bharti, A. K., Saxena, R. K., ... & Cook, D. R. (2012). Draft genome sequence of pigeon pea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. *Nature Biotechnology, 30*(1), 83–89.

[18] Wang, Y., Liu, Z., Wu, Z., Li, H., Wang, W., Cui, X., (2019). Genome-wide identification and expression analysis of GRAS family transcription factors in tea plant (Camellia sinensis). *Scientific Reports*, 9(1), 1–15.

[19] Zhao, Y., Zhang, Z., Gao, J., Wang, P., Hu, T., Wang, Z., et al. (2017). Arabidopsis duodecuple mutant of SnRK2s reveals the prominent role of SnRK2 in stress responses. *Plant Physiology*, 174(4), 2108–2120.