Biological Forum – An International Journal **15(6): 302-305(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

In-silico Analysis of SMXL Transcription Factor genes in Pigeon Pea (*Cajanus cajan* (L.) Millsp.)

Sreeshma N.[1], Manoj M.L.[1] and Nagendra K.S.[2]*
[1]Ph.D. Scholar, Division of Molecular Biology and Biotechnology, ICAR-IARI, New Delhi, India.
[2]National Professor, ICAR-NIPB, New Delhi, India.

(Corresponding author: Nagendra K.S.)*
(Received: 19 March 2023; Revised: 25 April 2023; Accepted: 04 May 2023; Published: 20 June 2023)
(Published by Research Trend)

ABSTRACT: Pigeon pea is a hardy crop useful for climate-resilient agriculture and nutritional security. Research on the shoot architecture and plant type of the crop will help produce high-yielding varieties suitable for mechanization. Finding genes to improve shoot architecture in pigeon pea is difficult because there is not a complete genome sequence or data on growth habits in different genotypes. Strigolactone (Sl) is a plant hormone involved in the root and shoot architecture. Understanding the Sl signalling pathway in pigeon pea will help to make varieties with the desired shoot architecture. The Sl signalling pathway involves the degradation of Suppressor of MAX2 Like (SMXL) genes, resulting in the expression of Sl-responsive genes. SMXLs are transcription repressors, and the presence of the RGKT motif is necessary for ubiquitination and degradation of this protein. In this study, seven SMXL genes present in the pigeon peagenome were identified and characterized using in silico methods. Two of the identified CcSMXL genes, namely CcSMXL3 and CcSMXL5, have the RGKT motif. Using the identified genes, the core population can be genotyped for the phenotype. Expression analysis of these genes in pigeon pea germplasm, together with phenotypevariation for shoot architecture, would help establish their association with the trait for utilization of these genes in pigeon pea improvement. Additionally, it will help to enhance the shoot architecture by using breeding and biotechnology tools.

Keywords: Pigeon pea, shoot architecture, Strigolactone, SMXL.

INTRODUCTION

Shoot branching is one of the key factors determining the shoot architecture or growth habit of dicotyledonous plants. Ideal plant type is an important area of research targeting higher harvest indexes, as exemplified by the deployment of semi-dwarfing genes in rice and wheat, ushering in the green revolution (Liu *et al*., 2020). The challenge of current agricultural research is the adoption of climate-resilient agriculture (CRA) and the realization of sustainable development goals. The adoption of climate-resilient crop varieties and climate-smart crop management practises are the two main pillars of CRA. Mechanization of smallholder farms demands varieties with suitable ideotypes (Prasad *et al.*, 2015). Pigeonpea is a naturally drought-tolerant pulse legume that is cultivated mostly by small-holder farmers in India under rain-fed agriculture with minimal inputs. It is essential to India's largely vegetarian population's food and nutritional security. Understanding the shoot architecture of pigeon pea is essential because it will help develop climate-resilient varieties needed for climate-smart agriculture (Dutta *et al*., 2022). Tall, medium, and dwarf heights, open and compact branching, early and late maturation periods, and open and compact branching are all phenotypes of currently available pigeon pea genetic resources and are mostly traditional varieties (Upadhyaya *et al.,* 2007). Understanding the underlying genetic components is critical for improving the shoot architecture for accelerated breeding for mechanized harvesting and pest management.

Strigolactone (Sl) is a plant hormone known to influence the root and shoot architecture of plants. D14 (DWARF14) is a hydrolase that acts as a Sl receptor in the Sl signalling pathway. D14 interacts with the SCF ubiquitin E3 ligase complex via D3/MAX2 (DWARF3/MORE AXILLARY BRANCHED 2), leading to degradation of D53/SMXLs (DWARF 53/MAX2 SUPPRESSOR LIKE 6, 7, and 8). The SMXLs are transcription repressors, and SMXL6 is an auto-regulator that binds to the promoters of SMXLs (Tal *et al*., 2022). When SMXLs degrade the repressors of transcription of Sl-responsive genes, target gene expression gets restored. The SMXL proteins have two Clp-N motifs, a P-ring motif, and an EAR motif. The RGKT motif is necessary for Sl signalling, as a mutation in this motif affects proteasomal degradation. The SMXL genes have been characterized in Arabidopsis, rice, and many other species. Arabidopsis thaliana has eight SMXL genes, and *Oryza sativa* has nine orthologous OsD53 genes. Out of these, AtSMXL 6, 7, and 8 are involved in Sl signalling. Light can regulate AtSMXL 6 and 7, whereas AtSMXL1 is involved in Karrikin signalling (Zhang *et*

al., 2022). Even though the genome sequence of pigeon pea has been available for over a decade (Singh *et al.*, 2012), its Sl signalling genes are not yet characterized. Since shoot architecture and branching are important yield-contributing traits in pigeon pea, this study was designed to characterise the SMXL transcription factor genes involved in Sl signalling in pigeon pea.

METHODOLOGY

The SMXL genes in the available pigeonpea genome were identified using NCBI tBlastn with the *A. thaliana* SMXL gene as a query. From the identified sequence hits, 30 Kb genomic sequences around the regions of similarity were used to predict genes using FGENESH (http://www.softberry.com/berry.phtml?topic=fgenesh &group=programs&subgroup=gfind). The online tools PHAMMER (https://www.ebi.ac.uk/Tools/hmmer/search/phmmer) and SUPERFAMILY (https://supfam.org/) were then used to identify domains. Both the RGKT and EAR (LxLxLx) motifs were manually verified. Based on their genomic and predicted CDS sequences, the structure of genes was predicted using GSDS 2.0 (http://gsds.gao-lab.org/). ExPasy's PprotPparam tool (https://web.expasy.org/protparam/) was utilized to calculate physiochemical properties. Twenty motifs were predicted using MEME SUIT with all parameters left at their default settings (https://meme-suite.org/meme/tools/meme). CLUSTALW was used to perform multiple sequence alignments of *A. thaliana*, *O. sativa*, and *C. cajan* SMXL genes available in MEGA XI software, and a phylogenetic tree was constructed using the neighbour joining method in MEGA XI. LOCALIZER (https://localizer.csiro.au/) was used to predict subcellular gene locations, and MULocDeep (https://mu-loc.org/) was used to predict sub-celluar gene locations. The functions of the discovered genes were annotated using Blast2Go.

RESULTS AND DISCUSSION

A. Gene organization and sub-cellular location of CcSMXL proteins

The available *C. cajan* genome sequence contains seven SMXL genes, randomly named CcSMXL1 to CcSMXL7 (Table 1). The existence of p loop-containing domains and Double Clp-N motifs was verified using SUPER FAMILY. We identified the EAR motif sequence LxLxL in CcSMXL3, 5, and 6; the LELCF motif in CcSMXL2 and CcSMXL7; and the LELSV motif in CcSMXL1 and CcSMXL4 (Fig. 1). Although LxLxL or DLNxxP is the dominant motif (Soundappan *et al.*, 2015), three CcSMXLs possess the sequence LCLCL, whereas four CcSMXL with EAR motifs have Ld/eLc/sf/v. Poplar also has variations in the SMXL motif sequence (Sun *et al.*, 2023). Despite the presence of EAR motif in the SMXLs, mutations do not impair SMXL functionality (Zhang *et al.*, 2022). Only two CcSMXLs, CcSMXL3 and CcSMXL5, have the RGKT motif, and they both share the same gene structure and motif structure (Fig. 2). D53 like/SMXL proteins are directed to protein degradation by Sl to prevent branching, and the RGKT motive is required for proteasomal degradation (Tal *et al.*, 2022). Sl signaling in pigeon pea may be mediated by CcSMXL3 and CcSMXL5. CcSMXL1 and CcSMXL4 have modified RGKT motif with amino acids replaced as R by M in CcSMXL1 and G by R in CcSMXL4. The Changse in amino acid sequences are also reported in Poplar (Sun *et al.*, 2023). The sequence of motifs is closer for a phylogenetic group. Three groups based on motif structure, i.e., CcSMXL3, CcSMXL5, and CcSMXL6, CcSMXL4, CcSMXL1, and CcSMXL7, are also closer in phylogenetic relationships (Fig. 2).

The CcSMXL genes are 3297–6326 bp long with two to five introns and 796–1126 amino acid residues. The predicted proteins have a molecular weight range of 89.21 to 124.66 and a predicted pI range of 6 to 7. The CcSMXL genes have negative grand average hydropathicity (GRAVY) values, indicating that they are hydrophilic (Table 1), supporting the idea that transcription factors are frequently found in the nucleoplasm rather than the membrane.

The location of the CcSMXL genes was predicted in the nucleus. Transit peptides for chloroplast localization were present in CcSMXL1, 2, 4, and 7, and these genes were predicted to be present in both the nucleus and chloroplast. There were nucleoli containing CcSMXL1, 3, 4.5, and 6. The nucleolus and nucleoplasm are predicted sites for CcSMXL4, while chromosomes are the localization sites for CcSMXL2 and 7. The location of transcription factors in the nucleolus or nucleoplasm and their association with the nucleolus and chromosomes are the basic characteristics of transcription factors.

B. Evolution and Phylogeny of the CcSMXL genes

A phylogenetic tree was constructed using 24 aligned sequences from three different plant species, including *Arabidopsis thaliana*, *Oryza sativa*, and *Cajanus cajan*, identifying four phylogenetic groups (Fig. 3). While Arabidopsis and rice SMLXs were present in all four groups, the pigeon pea CcSMXLs were restricted to groups 1 and 4 only. This under representation may be due to the lack of a complete reference genome sequence of pigeon pea. Except for CcSMXL2 and CcSMXL7, which were present in group 4, all other pigeon pea SMLX genes were present in group 1. The CcSMXL genes with RKGT motif or modified RGKT motif comes in Group 1. According to Blast 2go results, all group 1 CcSMXLs are ATP-binding and hydrolyzing proteins. The CcSMXL2 and CcSMXL7, which are hydrolases, are present together in group 4 (Fig. 3). CcSMXL2 and CcSMXL7 share similar motif structures, comparable molecular weights, numbers of amino acids, GRAVY, and pI to the other classes (Table 1, Fig. 1). This supports the hypothesis put forth by Moturu *et al.* (2018) that there is a close relationship between the phylogenetic class of the SMXL genes and their functional specialization. According to SUPER FAMILY, all CcSMXLs belong to the extended AAA-ATPase domain family.

Table 1: Physiochemical properties of CcSMXL genes.

Sr. No.	Genes Identified	No. of amino acid residues	Molecular weight (KDa)	Predicted pI	GRAVY
1.	CcSMXL1	1092	120.89	6.44	-0.321
2.	CcSMXL2	838	93.70	6.4	-0.397
3.	CcSMXL3	1094	121.04	6.65	-0.34
4.	CcSMXL4	1101	121.42	6.04	-0.339
5.	CcSMXL5	1126	124.66	6.75	-0.327
6.	CcSMXL6	1016	112.84	6.23	-0.393
7.	CcSMXL7	796	89.21	6.91	-0.392

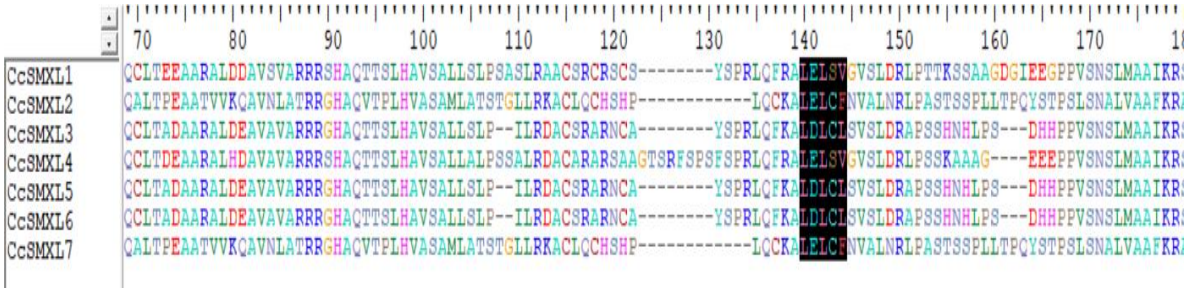

Fig. 1. Multiple sequence alignment of seven CcSMXLs showing the EAR motif position marked with a dark shade.

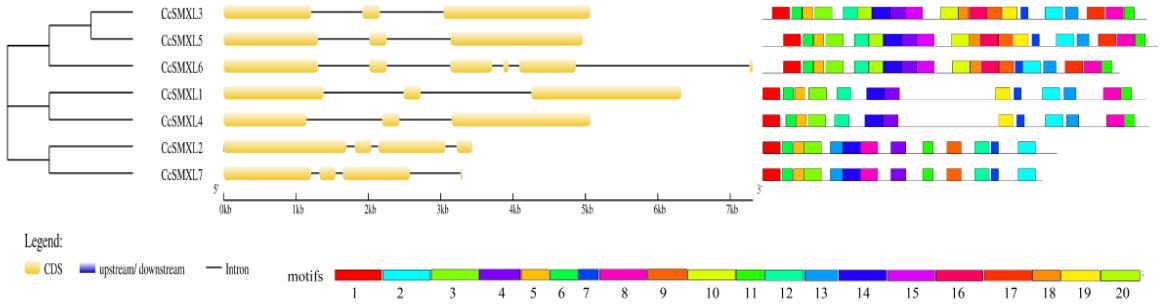

Fig. 2. Phylogenetic relationship, gene structure, and amino acid motifs in the pigeon pea CcSMXL genes.

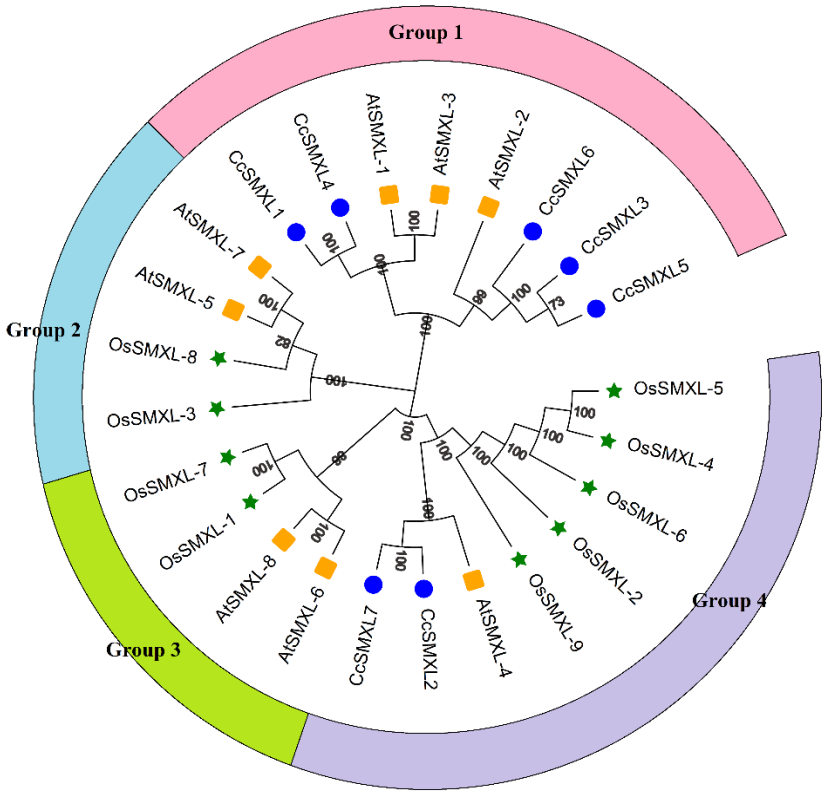

Fig. 3. Phylogenetic tree of 24 SMXL genes from *A. thalliana*, *O. sativa* and *C. cajan.* Yellow square-Arabidopsis, Green star- Rice and Blue circle- Pigeon pea genes.

CONCLUSIONS

In the available genome sequence of pigeonpea, seven CcSMXL genes were identified. The RGKT motif, required for Sl signalling, is present in only two SMXL genes, namely CcSMXL3 and CcSMXL5. The CcSMXL genes appear to have a common phylogenetic ancestry, motif sequences, and functions.

FUTURE SCOPE

Further research into the Sl signalling pathway and SMXL genes will help in crop improvement. Designing crop breeding programs utilizing breeding and genetic engineering will be made easier by understanding the relationship between CcSMXLs and the architecture of shoots and roots in pigeon pea germplasm.

Acknowledgments. We would like to express our gratitude to the Dean, ICAR-IARI, and Director, ICAR-NIPB, for giving us all the resources and assistance that we needed to complete the study. The first author expresses gratitude to ICAR- IARI and DBT-JRF for fellowship and to Professor, Division of molecular Biology and Biotechnology.
Conflict of Interest. None.

REFERENCES

Dutta, A., Trivedi, A., Nath, C. P., Gupta, D. S. & Hazra, K. K. (2022). A comprehensive review on grain legumes as climate-smart crops: Challenges and prospects. *Environmental Challenges*, 100479.

Liu, S., Zhang, M., Feng, F. & Tian, Z. (2020). Toward a "green revolution" for soybean. *Molecular plant*, *13*(5), 688-697.

Moturu, T. R., Thula, S., Singh, R. K., Nodzyński, T., Vařeková, R. S., Friml, J. & Simon, S. (2018). Molecular evolution and diversification of the SMXL gene family. *Journal of Experimental Botany*, *69*(9), 2367-2378.

Prasad, Y. G., Ch Srinivasarao, S. Dixit, M. Maheswari, J. V. N. S. Prasad, B. Venkateswarlu, and A. K. Sikka. (2015). Evidences from Farmer Participatory Technology Demonstrations to Combat Increasing Climate Uncertainty in Rainfed Agriculture in India." Procedia Environmental Sciences, Agriculture and Climate Change - Adapting Crops to Increased Uncertainty (AGRI 2015), *29*, 291–92.

Singh, N. K., Gupta, D. K., Jayaswal, P. K., Mahato, A. K., Dutta, S., Singh, S. & Sharma, T. R. (2012). The first draft of the pigeon pea genome sequence. *Journal of plant biochemistry and biotechnology*, *21*, 98-112.

Soundappan, I., Bennett, T., Morffy, N., Liang, Y., Stanga, J. P., Abbas, A. & Nelson, D. C. (2015). SMAX1-LIKE/D53 family members enable distinct MAX2-dependent responses to strigolactones and karrikins in Arabidopsis. *The Plant Cell*, *27*(11), 3143-3159.

Sun, M., Wang, D., Liu, C., Liu, Y., Niu, M., Wang, J., & Li, J. (2023). Genome-wide identification and analysis of the SUPPRESSOR of MAX2 1-LIKE gene family and its interaction with DWARF14 in poplar. *BMC Plant Biology*, *23*(1), 1-16.

Tal, L., Palayam, M., Ron, M., Young, A., Britt, A. & Shabek, N. (2022). A conformational switch in the SCF-D3/MAX2 ubiquitin ligase facilitates strigolactone signalling. *Nature Plants*, *8*(5), 561-573.

Upadhyaya, H. D., Reddy, K. N., Gowda, C. L. L. & Singh, S. (2007). Phenotypic diversity in the pigeonpea (*Cajanus cajan*) core collection. *Genetic Resources and Crop Evolution*, *54*, 1167-1184.

Zhang, H., Wang, L., Gao, Y., Guo, Y., Zheng, N., Xu, X. & Yang, W. (2022). Genome-Wide Identification of SMXL Gene Family in Soybean and Expression Analysis of GmSMXLs under Shade Stress. *Plants*, *11*(18), 2410.

How to cite this article: Sreeshma N., Manoj M.L. and Nagendra K.S. (2023). *In-silico* Analysis of SMXL Transcription Factor genes in Pigeon Pea (*Cajanus cajan* (L.) Millsp.). *Biological Forum – An International Journal*, *15*(6): 302-305.