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Genome-Wide Identification and Characterization of the Strigolactone (SL) Pathway and Associated Genes in Sorghum

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Abstract

Strigolactones (SLs) are a novel class of plant hormones that play critical roles in regulating developmental processes and stress tolerance. Even though the SL-related genes have been identified and characterized in model plants such as *Arabidopsis* and rice, characterization of SL-related genes in crop plants, particularly dry land crops like sorghum (*Sorghum bicolor*), have yet to be fully explored. In this study, the SL-pathway and associated genes and their expression patterns under abiotic stress were systematically identified and characterized in the sorghum. This study identified the SL pathway and associated genes, including biosynthesis (*D27*, *CCD7*, *CCD8*, *MAX1* and *LBO*) and signaling (*D14*, *MAX2*, *D53*). Phylogenetic analysis revealed that all SL-related genes are highly conserved among plant species. Furthermore, the expression analysis showed that most SL-related genes are involved in cold, drought and simulated drought/ABA stress response. These findings provide valuable information for further investigation and functional characterization of SL-biosynthetic and signaling genes in response to abiotic stresses in sorghum.

Keywords: Sorghum, strigolactones, signaling genes; abiotic stress

Introduction

Sorghum is a cereal grain crop that belongs to the family Poaceae and is native to Africa. It is widely cultivated worldwide and utilized for many uses, including food, animal feed, fuel, and industrial applications. *Sorghum* is a versatile crop that can grow in various environments, from dry land areas with low rainfall to high humidity and precipitation. It is also tolerant to heat, drought, and poor soil conditions, making it a valuable crop in regions where other crops struggle to grow. Sorghum is often used as a drought-tolerant and alternative to maize in regions with water scarcity, such as parts of Sub-Saharan Africa (SSA). Plants have developed a variety of adaptations in their metabolism, physiology, and biochemical systems to adapt to the osmotic stress they are subjected to because of their sessile nature. Previous research has demonstrated that certain plant hormones, including auxin (IAA) (Bielach et al., 2017), brassinolide (BR) (Talaat & Shawky, 2016), abscisic acid (ABA) (Fricke et al., 2004) and strigolactones (SLs) (Zulfiqar et al., 2021, Kaniganti et al., 2022) gibberellin (GA) (Colebrook et al., 2014), play important roles in response to abiotic stress.

SLs are a novel class of plant hormones

first discovered as a root secretory chemical that promoted the seed development of parasitic weeds like *Striga lutea* (Cook et al., 1966). It has been demonstrated that SLs are crucial in regulating root architecture, suppressing shoot branching, and enhancing leaf senescence (Umehara et al., 2008). The development of crop varieties resistant to abiotic and biotic stress can be achieved by manipulating components of strigolactone (SL) biosynthesis and signaling. Over 20 SL and SL-like compounds have been identified in the root exudates of various plant species. Understanding SL biosynthesis is crucial for applying this knowledge effectively in modern agriculture, leading to more resilient crops and sustainable farming practices. SL biosynthesis pathway involves different enzymes, mainly D27: a *cis/trans*-carotene isomerase, carotenoid cleavage dioxygenase 7 and 8 (CCD7, CCD8), more axillary growth 1 (MAX1): a cytochrome P450 monooxygenase and lateral branching oxidoreductase (LBO): an oxidoreductase-like enzyme (Lopez-Obando et al., 2015; Brewer et al., 2016). *DWARF27* (D27) first mediated the isomerization of trans-carotene to 9-*cis*-carotene. Next, 9-*cis*-carotene is transformed to 9-*cis*-apo-10′-carotenol, and CCD8 is then processed into the SLs precursor carlactone (CL). The biosynthesis pathway of CL from trans-carotene is identical across plant species. However, the biosynthesis pathway from CL to active SLs may vary from plant-to-plant species. In *Arabidopsis*, the biosynthesis of SLs involves several steps. The precursor compound, carlactone (CL), is catalyzed by the enzyme MAX1 to form carlactonoic acid (CLA). CLA is then methylated by a methyl transferase to produce methyl carlactonoate (MeCLA). Finally, MeCLA is transformed into a bioactive strigolactone through the action of the enzyme LBO. In rice, however, CL is transformed into CLA by the MAX1 homolog Os900 (CYP711A2), which is then turned into 4-deoxyorobanchol (4DO) by CYP711A2. Then, another MAX1 homolog (CYP711A3) catalyzed 4DO to generate orobanchol, which possesses SL activity (Wu et al., 2019). Significant progress has been made now in understanding the SL signal transduction pathway. This pathway involves several crucial genes, including *DWARF14* (*D14*) (Yao et al., 2016), *DWARF 3* (*D3*)/*More Axillary Growth 2* (*MAX2*) (Stirnberg et al., 2007), *DWARF 53* (*D53*)/*SMXLs* (Zhou et al., 2013), *TOPLESS* (*TPL*)*-related protein two* (*TPR2*), and *Ideal Plant Architecture 1* (*IPA1*) (Song et al., 2017). D14 acts as the SL receptor and plays a pivotal role in binding and hydrolyzing SL. MAX2 is part of the SCF complex, which degrades the nuclear-localized repressor D53 upon SL binding, activating downstream SL signaling. Conversely, in the absence of SLs, D53 forms a complex with *TPR2* and *IPA1* to repress the expression of IPA1-regulated genes, resulting in no SL response.

Recent studies have also highlighted the involvement of SL-related genes in plant responses to abiotic stress. For instance, in Arabidopsis, mutants like *max2*, *max3*, and *max4* demonstrated increased sensitivity to salt and drought stress compared to wild-type plants (Ha et al., 2014). Specific proteins, such as SMXL6, SMXL7, and SMXL8, negatively regulated drought tolerance in Arabidopsis (Li et al., 2020). These investigations reveal the intricate connections between SL signal transduction and plant stress tolerance. The findings provide valuable insights that could contribute to the development of stress-resistant crop varieties in the future.

The present study investigates SL-related genes in sorghum, including their gene structures, phylogenetic relationships and chromosomal distributions. Additionally, we examined the expression patterns of these genes in different organs and under diverse abiotic stress conditions. The findings from this research offer valuable insights into the characteristics and regulatory mechanisms of SL biosynthetic and signalling genes in sorghum. The data generated will serve as a foundation for future functional analyses of these genes, facilitating a deeper understanding of their roles in plant growth, development, and response to

environmental stresses. Overall, this study advances knowledge regarding SL-related genes in sorghum, opening new avenues for research in crop improvement and stress tolerance in agriculture.

Materials and Methods

Sequence extraction for SL-related genes

To identify the genes involved in SL biosynthesis and signaling, genes and the related information for sorghum were retrieved from Sorghum genome assembly v3.1.1 from the Phytozome database (https://phytozome-next.jgi.doe.gov/info/Sbicolor_v3_1_1). SL biosynthetic genes and the related information of rice, maize, Arabidopsis, and soybean were obtained from previous studies (Wang et al., 2017; Yoneyama et al., 2018; Wu et al., 2019; Qiao et al., 2020). The rice, maize, Arabidopsis, and soybean protein sequences were used as query sequences using the sorghum protein file as the subject file in TB tools (Chen et al., 2020). Redundant sequences were removed from the obtained sequences, and the occurrence of a specific domain was confirmed using InterPro (https://www.ebi.ac.uk/interpro/search/sequence/). The isoelectric point (pI) and molecular weight (MW) of the identified SL-related proteins were predicted using the ExPASy online software (https://web.expasy.org/protparam/). The exPASy tool was used to calculate the various physicochemical properties of proteins. Additionally, to determine the subcellular localization of sorghum SL-related, we used Cell-PLoc 2.0 software (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/).

Sequence analysis

Multiple Sequence Alignment (MSA) of SL-related genes from sorghum, rice, maize, Arabidopsis, and soybean was performed using the Clustal W program. The evolutionary relationships between the SL-related genes of sorghum and other species were performed using the Neighbour-Joining (NJ) method with 1000 bootstraps in MEGA 11 software. Conserved motifs were predicted using Multiple EM for Motif Elicitation (MEME) (https://meme-suite.org/meme/) online tool by comparison with rice, maize, Arabidopsis, and soybean. Up to 15 motifs were permitted in the tool with other parameters at default settings.

Synteny analysis of SL-related genes

To establish a syntenic relationship between SL-related genes of sorghum along with rice and maize, a Multiple Collinearity Scan toolkit (MCScanX) program with default parameters was employed. Syntenic maps based on the MCScanX analysis were generated using the Dual Synteny Plotter tool of TBtools Software (https://github.com/CJ-Chen/TBtools). The synonymous substitution rates (Ks) and non-synonymous substitution rates (Ka) of the obtained syntenic pairs were calculated using the Simple Ka/Ks calculator tool of TBtools software. The obtained Ka/Ks ratios were then used to determine the selection pressure of the syntenic pairs of SL-related genes between sorghum and rice and sorghum and maize.

Chromosomal location and Intron-Exon prediction

Chromosomal location and position of the SL-related genes in sorghum were obtained from the Phytozome database. The chromosomal map was generated using the MapGene2Chromosome (MG2C) v2.0 (24). The information regarding the intron-exon junctions, such as genomic sequence and coding DNA sequence of SL-related genes, was obtained from the Phytozome database. The intron-exon junctions of SL-related genes were represented by using Gene Structure Display Server (GSDS) 2.0 (25).

Expression profiling of Sorghum SL-related genes

Genevestigator platform utilized to decipher the expression profiles of genes subjected to environmental stress conditions were extracted from the sorghum array and used for cluster analysis. Further, a heat map of expression

profiling was generated by utilizing the hierarchical clustering tool of the Genevestigator platform (https://genevestigator.com/gv/) (26). The mRNA-seq data were used for analysis. The data are available for all 12 genes for two stress conditions (cold and drought) in three tissues (root, shoot, and leaf) and four developmental stages (milk stage, seedling stage, tillering stage, and flowering stage). Heat maps were generated separately using hierarchical clustering.

Results

Identification of SL-related genes

SL biosynthetic genes have been identified in some plant species, such as *Arabidopsis*, rice, and soybean (Waters et al., 2017; Qiao et al., 2020). These protein sequences were used as queries to retrieve homologous proteins in sorghum genome databases. The list of analysed proteins used in this study is provided in supplementary table 1. One of *D27*, *CCD7*, *CCD8*, and *LBO* genes and 4 of *Max 1* were obtained from the sorghum genome by removing incomplete and redundant sequences. Detailed information regarding the identified SL biosynthetic proteins is provided in Table 1, including the gene name, gene ID, length of coding DNA sequence (CDS), length of protein sequence, molecular weight (MW), theoretical isoelectric point (pI) and subcellular localization.

Table 1: Physicochemical properties of sorghum SL biosynthetic and signaling genes.

Name	Gene ID	Phytozome description	Chromosome	CDS (bp)	Protein length (AA)	Molecular weight (KDa)	Isoelectric point(pI)	No. of Introns/ Exons	Subcellular localization
SL biosynthesis genes									
SbD27	Sobic.005G168200	beta-carotene isomerase D27	5	879	293	32325.36	8.72	6/7	Nucleus
SbCCD8	Sobic.003G293600	Carotenoid cleavage dioxygenase 8	3	1740	580	63075.49	6.61	3/4	Chloroplast
SbCCD7	Sobic.006G170300	Carotenoid cleavage dioxygenase 7	6	1890	630	69973.19	8.92	6/7	Chloroplast
SbMAX1a	Sobic.010G170400	Cytochrome P450 monooxygenase CYP711A12	10	1623	541	58970.95	8.99	4/5	Endoplasmic reticulum
SbMAX1b	Sobic.003G269500	Cytochrome P450 monooxygenase CYP711A12	3	1644	548	61135.6	8.69	4/5	Endoplasmic reticulum
SbMAX1c	Sobic.004G095500	Cytochrome P450 monooxygenase CYP711A12	4	1638	546	60870.32	9.14	2/3	Endoplasmic reticulum
SbMAX1d	Sobic.003G269500	Cytochrome P450 monooxygenase CYP711A12	3	1644	548	61135.60	8.69	2/3	Endoplasmic reticulum
SbLBO	Sobic.003G418000	Lateral branching oxidoreductase	3	1113	371	41259	5.29	2/3	Cytoplasm
SL signaling genes									
SbD53a	Sobic.005G002400	Dwarf 53	5	3255	1085	117911.24	6.44	2/3	Chloroplast
SbD53b	Sobic.008G002400	Dwarf 53	8	3387	1129	122742.71	6.76	2/3	Chloroplast
SbMAX2	Sobic.010G043000	F-box/LRR-repeat MAX2 homolog	10	2103	701	76775.6	5.22	0/1	Nucleus
SL receptor genes									
SbD14	Sobic.003G206900	α/β hydrolyzyme	1	897	299	31887.94	4.5	2/3	Cell membrane

The phylogenetic trees were constructed using the protein sequences of these genes with Arabidopsis, rice, soybean and maize to evaluate the evolutionary relationships of sorghum SL biosynthetic proteins with other plants. Phylogenetic analysis indicated

that the sorghum D27 (Sobic.005G168200) clustered with monocotyledonous plants like rice and wheat, suggesting its functional similarity (Fig 1). MAX1 is a cytochrome P450 monooxygenase synthesizing carlactonic acid (CLA) from carlactone (CL). CCD7 and CCD8 are carotenoid cleavage dioxygenases involved in SL biosynthesis. SbCCD7 and SbCCD8 were identified in sorghum, showing high similarity to their respective homologues in other plant species. The motifs in CCD7 and CCD8 were found to be conserved, except for a few variations. LBO is an oxidoreductase-like enzyme involved in the later stages of SL biosynthesis. SbLBO was identified in sorghum, clustering with other LBO proteins (Fig 1).

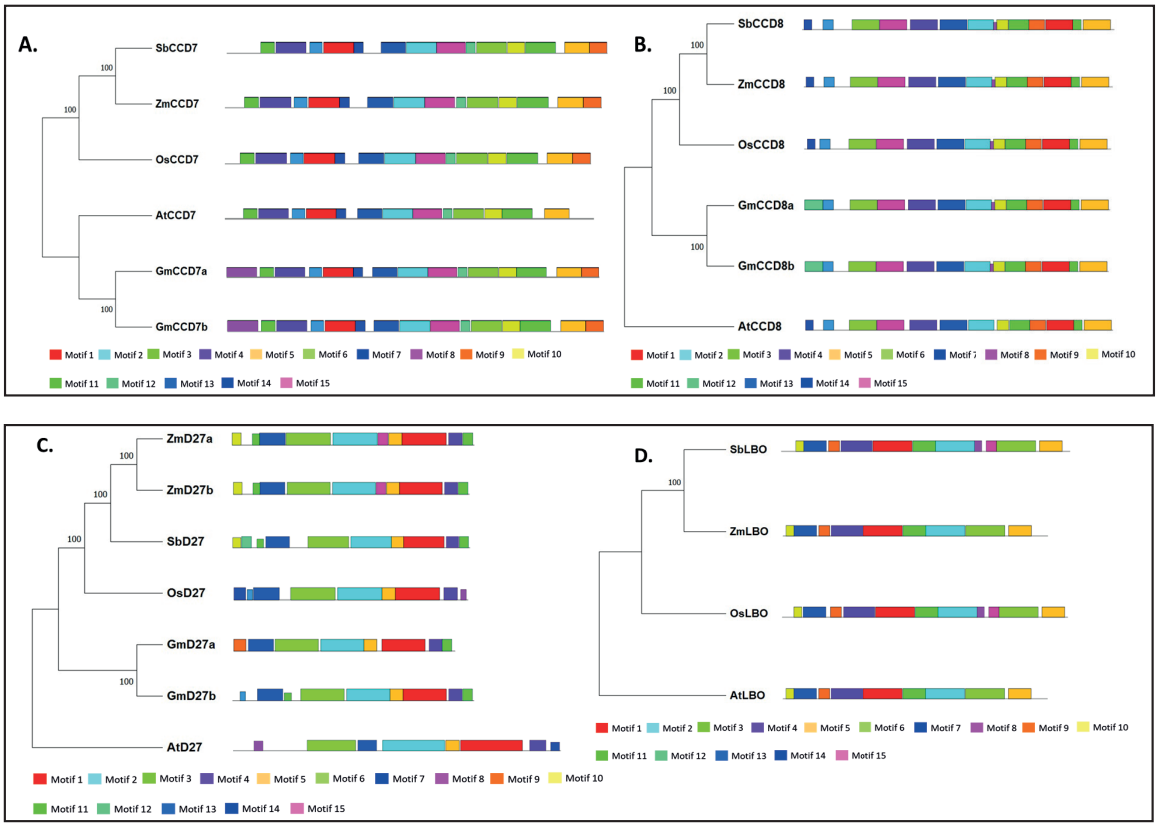

Figure 1: Phylogenetic tree and conserved motif analysis for the SL-related genes. A. CCD7 proteins B. CCD8 proteins C. D27 proteins D. LBO proteins. The phylogenetic relationship was determined between Sorghum (Sorghum bicolor), Soybean (Glycine max), Arabidopsis (Arabidopsis thaliana), Rice (Oryza sativa) and Maize (Zea mays). Motif analysis was done using the MEME online tool; up to 15 motifs were identified and represented in coloured boxes. The grey lines represent non-conserved motifs.

Several conserved motifs were found in LBO proteins across species. Four MAX1-like proteins (MAX1a, MAX1b, MAX1c, and MAX1d) were identified in sorghum, with three of them clustering with maize MAX1 proteins and the fourth one forming a separate cluster with the CYP711 clan. Motif analysis revealed conserved motifs in MAX1 proteins, except for motifs 8 and 12 (Fig 2).

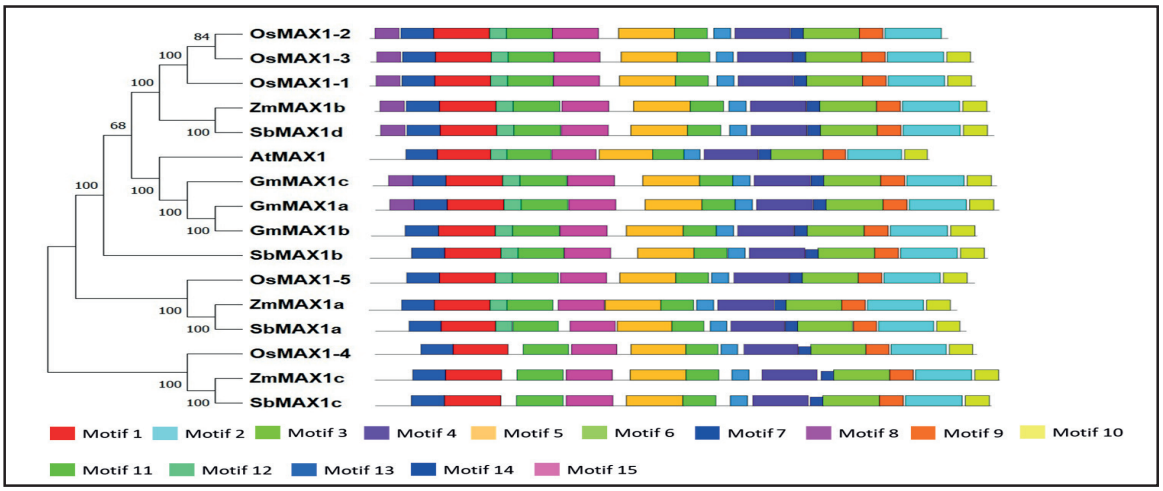

Figure 2: Phylogenetic tree and conserved motif analysis of MAX1 proteins. The phylogenetic relationship was determined between Sorghum (Sorghum bicolor), Soybean (Glycine max), Arabidopsis (Arabidopsis thaliana), Rice (Oryza sativa) and Maize (Zea mays). Motif analysis was done using the MEME online tool; up to 15 motifs were identified and represented in coloured boxes. The grey lines represent non-conserved motifs.

The SL signaling pathway genes identified in sorghum include *MAX2* (*D3*), *D53*, and *D14*. *SbMAX2* (Sobic.010G043000) was identified as a *MAX2* gene homolog involved in SL signal transduction. Two D53-like proteins, SbD53a (Sobic.005G002400) and SbD53b (Sobic.008G002400), were identified in sorghum, acting as repressors of SL signaling. SbD14 (Sobic.001G465100) was identified as a homolog of the D14 gene, which plays a crucial role in SL signal perception (Fig 3).

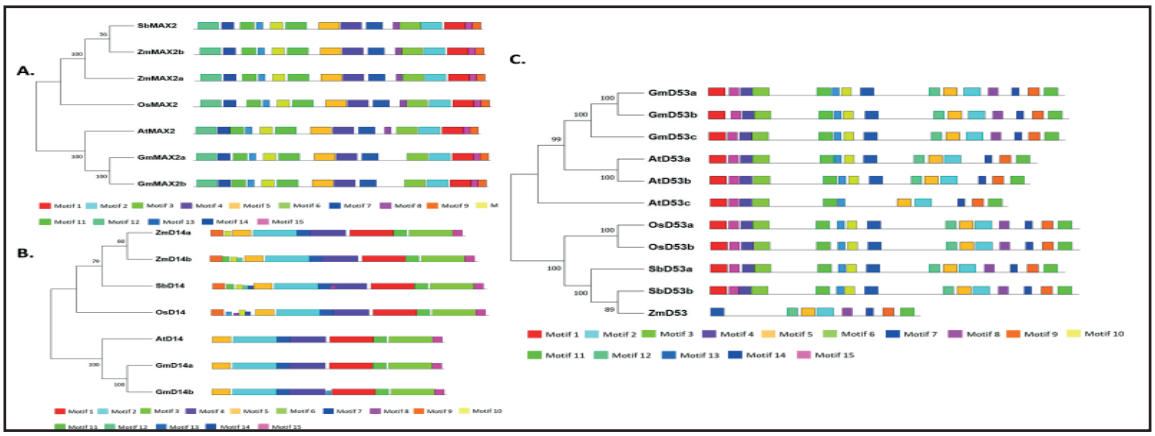

Figure 3: Phylogenetic tree and conserved motif analysis SL-related genes. A. MAX2 proteins, B. D14 proteins and C. D53 proteins. The phylogenetic relationship was determined between Sorghum (Sorghum bicolor), Soybean (Glycine max), Arabidopsis (Arabidopsis thaliana), Rice (Oryza sativa) and Maize (Zea mays). Motif analysis was done employing MEME online tool; up to 15 motifs were identified and represented in the form of coloured boxes. The grey lines represent non-conserved motifs.

Syntenic analysis of SL-related sorghum genes with rice and maize

To better understand the evolutionary relationship between SL-related genes of sorghum and other plant species, including rice and maize, comparative syntenic maps were generated between sorghum and rice and sorghum and maize, specifically emphasizing SL-related genes (Fig 4). Fourteen syntenic pairs were obtained for SL-related genes of sorghum in rice. Except for *SbMAX1b* and *SbD27*, all the other sorghum genes displayed one or more orthologous pairs in rice. The detailed list of syntenic pairs of sorghum and rice is given in the supplementary table 2. According to the syntenic analysis, duplication events for both *SbD53a* and *SbD53b* genes were observed in rice in which the rice genome had two copies like SbD53a (LOC_Os11g01330 and LOC_Os12g01360) and D53b (LOC_Os12g01360 and LOC_Os11g01330). Similarly, gene duplication events were also observed for two more sorghum genes, MAX1a (LOC_Os06g36920 and LOC_Os02g12890 in rice) and MAX1c (LOC_Os06g36920 and LOC_Os02g12890 in rice) (Fig 4, supplementary table 2).

In maize, 13 syntenic pairs were noticed for the sorghum SL-related genes. All the sorghum SL-related genes had one or more syntenic pairs in the maize genome (Fig 4). Gene duplication events were also observed in the maize genome, and SbD14 was observed to have two copies in the maize genome (Zm00001d048146 and Zm00001d028094). Similar to rice, maize also showed gene duplication for MAX1a (Zm00001d053569 and Zm00001d046207 in maize) and MAX1c (Zm00001d053569 and Zm00001d046207 in maize) (Fig 4, supplementary table 3). The detailed list of syntenic pairs between sorghum and maize is in supplementary file 3. To better understand the type of evolution followed by these gene pairs and the evolutionary constraint acting on them, Ka/Ks ratios were calculated for these gene pairs (supplementary tables 4 and 5). All the Ka/Ks ratios showed less than one value, suggesting that the sorghum SL-related genes have undergone a purifying selection process.

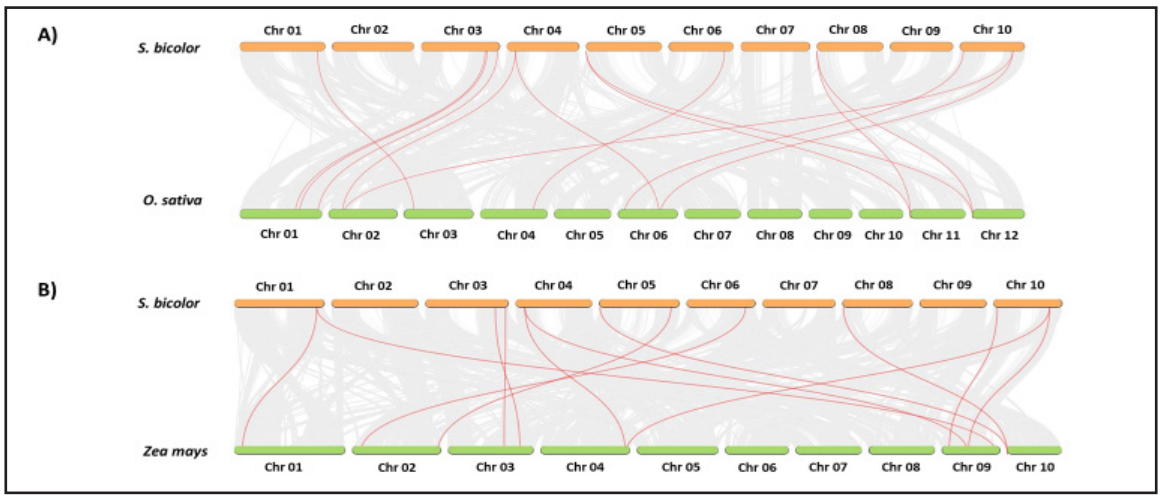

Figure 4: Synteny analysis of SL-related genes between Sorghum and two representative species. A. Synteny analysis of SL-related genes between Sorghum and rice B. Synteny analysis of SL-related genes between sorghum and maize. Grey lines in the background represent all collinear blocks between sorghum and the considered plant species, while the red lines highlight the syntenic SL-related gene pairs.

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Chromosomal location and intron-exon junctions of SL-related genes

A chromosomal location map was generated to investigate the distribution of SL-related genes across the chromosomes of the sorghum genome (Fig 5). The chromosomal map suggested the distribution of SL pathway-related genes across all sorghum chromosomes except for chromosomes 2 and 9. Multiple copies of the *SbMAX1* gene were distributed on different chromosomes of the sorghum genome, with *SbMAX1b* and *SbMAX1d* present on chromosome 3, *SbMAX1c* on chromosome 3 and SbMAX1a on chromosome 10. Like SbMAX1a, SbD53 was also present in two copies in the sorghum genome, with one of its copies, SbD53a, present on chromosome 5 and SbD53b on chromosome 8 (Fig 5).

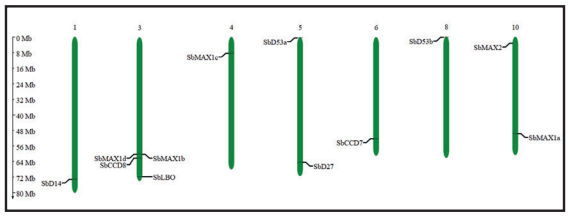

Figure 5: Location of SL-related genes on chromosomes. At the apex of each bar is the name of the chromosome. The length of chromosomes is represented in megabases (Mb).

The structure of introns/exons and UTRs of *strigolactone* genes were determined by aligning genomic and full-length cDNA sequences employing GSDS software (Fig 6).

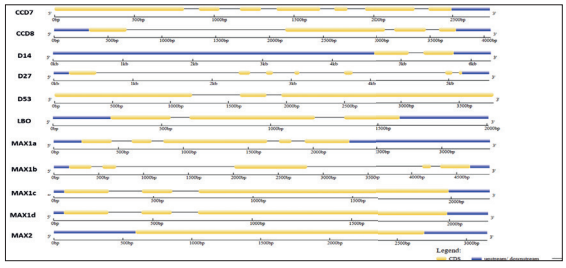

Figure 6: Intron-Exon structures of the sorghum SL-related genes. Yellow lines represent Coding DNA regions, Blue lines represent upstream/downstream regions and grey lines represent introns.

Digital expression profiling of SL pathway-related genes in sorghum in different tissues and abiotic stress conditions

Expression of SL pathway-related genes was studied at the transcriptional level in different tissues and abiotic stress conditions (cold, drought and drought stress induced by ABA treatment). SL biosynthesis genes (D27, CCD7, CCD8, MAX1 and LBO) have higher expression levels among the anatomical parts (shoot apical meristem, internode, floral apical meristem, floret, panicle, flag leaf, leaf, caryopsis, and embryo) than signaling genes (D14, MAX2, D53) (Fig 7A). The expression profiles of these genes were also analyzed at different plant developmental stages (germination, stem elongation, booting, flowering, tillering, heading, milk, dough, maturity, and seedling). Interestingly, all the SL biosynthesis genes have lower expression levels than signaling genes in all developmental stages (Fig 7B). Furthermore, under drought stress, six sorghum SL- genes, SbD27, SbCCD7, SbCCD8, SbMAX1, and SbMAX2, showed significantly similar expression levels in leaf when compared with control, whereas SL-gene SbD14 had lower expression levels in treated samples but SbLBO, SbMAX2 and SbD53 have similar expression pattern compared to that of control (Fig 7C). Under drought stress simulated by ABA treatment, increased expression levels were observed in the root compared to the shoot. SL biosynthesis genes SbCCD7, SbCCD8, SbMAX1, and SbMAX2 have not shown any expression in root tissues like that of control, whereas SbD27 has shown the optimal level of expression. SL-signaling genes have shown reduced expression levels in both root and shoot when compared with controls (Fig. 7D). Under cold stress, sorghum SL-biosynthesis gene expressions were upregulated in leaves compared to SL signaling genes (Fig 7E).

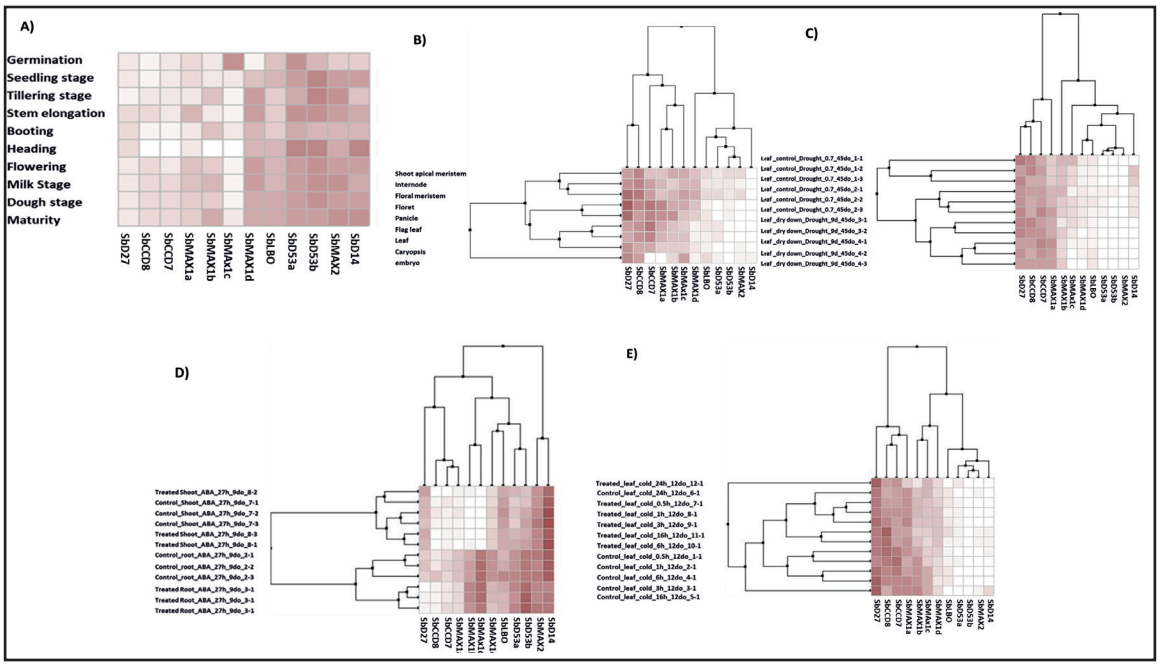

Figure 7: Expression profiling of SL-related genes in sorghum. A). The expression pattern analysis in different anatomical parts of sorghum. B) The expression pattern in different developmental parts of sorghum. C) The expression pattern under drought stress. D)The expression pattern analysis in root and shoot under simulated drought/ABA stress. E) The expression pattern in leaves under cold stress.

Discussion

In recent years, there has been an increasing scientific interest in the novel class of plant hormones called strigolactones (SLs). They have been found to play critical roles in plant development and responses to abiotic stresses (Cooper et al., 2018; Min et al., 2019; Zheng et al., 2021). While SL biosynthetic and signaling genes have been identified in several plant species, such as rice, *Arabidopsis*, soybean, and maize (Waters et al., 2017; Wu et al., 2019; Qiao et al., 2020), their presence and functions in sorghum have remained largely unexplored. Therefore, this study aimed to fill this knowledge gap by identifying SL pathway-related genes in the sorghum genome and investigating their potential roles in plant development and abiotic stress responses. The findings from this study shed light on the strigolactone (SL) biosynthesis and signaling pathways in sorghum and their evolutionary relationships with rice and maize. Identifying SL-related genes, including *D27*, *MAX1*, *CCD7*, *CCD8*, *LBO*, *MAX2*, *D53*, and *D14*, provides valuable information for understanding the molecular mechanisms underlying SL-mediated processes in sorghum. Using sequence similarity searches, domain analysis, motif conservation, and phylogenetic tree construction ensures robust identification and classification of these genes.

One of the significant findings is the identification of multiple MAX1-like proteins (MAX1a, MAX1b, MAX1c, and MAX1d) in sorghum. The clustering of three MAX1 proteins with maize MAX1 proteins and the fourth one forming a separate cluster with the CYP711 clan suggests gene duplication events and functional divergence within the MAX1 family during the evolutionary history of these species (Wu et al., 2019). The conserved motifs in MAX1 pro-

teins, except for motifs 8 and 12, indicate their functional importance in SL biosynthesis. The presence of two D53-like proteins, SbD53a and SbD53b, in sorghum and the gene duplication events observed in rice (LOC_Os11g01330 and LOC_Os12g01360) suggest the potential significance of these genes in SL signaling regulation. Additionally, identifying SbD14 as a homolog of the D14 gene involved in SL signal perception indicates the conservation of the SL signaling pathway in sorghum. The syntenic analysis between sorghum, rice, and maize provides valuable insights into the evolutionary relationships of SL-related genes among these cereal crops. The observed gene duplications in MAX1 and D53 genes in rice and maize genomes suggest that these genes might have undergone functional divergence after duplication events, leading to potential differences in their roles in SL-mediated processes (Ha et al., 2014). This information could be valuable for further research into regulating SL biosynthesis and signaling pathways under different environmental conditions.

The expression profiling of SL-related genes in various tissues and developmental stages provides a better understanding of their tissue-specific and developmental stage-specific regulation. Moreover, the upregulation of some SL-related genes in response to cold and drought stress indicates their potential role in stress response pathways, suggesting their importance in enhancing sorghum's resilience to adverse environmental conditions (Cardinale et al., 2018; Min et al., 2019), and some SL biosynthetic and signaling genes have been implicated in plant abiotic stress responses (An et al., 2016). For instance, Arabidopsis has demonstrated that AtMAX2, AtCCD7, and AtCCD8 positively regulate plant responses to salt and drought stresses (Ha et al., 2014).

Moreover, the expression analysis conducted in this study showed that eight sorghum SL-related genes were upregulated in both roots and leaves under cold, drought and simulated drought/ABA stress, indicating their involvement in stress response. Additionally, six genes (SbD27, SbCCD7, SbCCD8, SbMAX1, SbMAX2, and SbD53) exhibited significantly increased expression in roots and leaves under drought stress conditions. On the other hand, *SbLBO* and *SbD14* displayed a downregulated expression under drought stress induced by ABA treatment. These findings highlight the potential importance of the identified SL biosynthetic and signaling genes in sorghum's response to abiotic stresses. This study successfully identified SL pathway-related genes in the sorghum genome and shed light on their potential roles in abiotic stress responses. The conservation of motifs and the observed expression patterns under different stress conditions provide compelling evidence for the functional relevance of these genes.

Further research is needed to elucidate the precise mechanisms by which SLs and their associated genes contribute to stress tolerance in sorghum and other plant species. Understanding the roles of SL-related genes in stress responses can have significant implications for crop improvement strategies. Manipulating these genes or their regulatory pathways could enhance the stress tolerance of crops, leading to increased yield and productivity under challenging environmental conditions. Moreover, the knowledge gained from this study can also be applied to other crops, providing potential targets for genetic engineering or breeding approaches to improve abiotic stress resistance across various crop plant species.

Conclusions

This study successfully identified SL pathway-related genes systematically in the sorghum genome. These include five biosynthetic genes, three signaling genes, and a reporter gene. Importantly, all these genes are highly conserved among plant species, indicating their evolutionary significance. Furthermore,

the expression analysis revealed that these SL-related genes are differentially expressed in response to abiotic stresses in sorghum, highlighting their important regulatory roles in stress adaptation. These findings provide valuable insights for further research and functional analysis of the sorghum SL biosynthetic and signaling genes to abiotic stress responses.

Contributions

PSR designed the experiments. SK and MK carried out the bioinformatics analysis. SK, MK and PSR have written the initial draft. PSR and PBK have refined the manuscript. All authors have read and approved the manuscript.

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