Research Article

Genome-wide characterization and expression of the APETALA2 (AP2) gene family in *Glycine max* L. reveal its importance in cold stress responses modulated by melatonin application.

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ABSTRACT

The soybean (*Glycine max* L.) is the world's most significant oilseed crop in terms of total production and international trade. Determining the genetic composition of soybeans is essential for identifying their characteristics, enhancing crop productivity and quality, and creating sustainable farming methods. AP2/ERF transcription factors regulate the responses against biotic and abiotic factors in the plants. However, little investigation of the AP2 family has been conducted in soybean. This study aims to introduce the expression of AP2 genes. A total of 50 nonredundant AP2 genes were analyzed by using a systematic genome-wide analysis. These genes were distributed on 19 chromosomes except chromosome number 20 that have no AP2 gene. Analysis of gene structure confirmed the presence of 2-10 introns in the AP2 family. In addition to the signature AP2 domain being completely conserved, 19 other domains were discovered to be conserved in a group-specific way. Synteny analysis expressed an intensive resemblance of orthologous regions between soybean and *Arabidopsis thaliana*. *In-silico* expression study based on transcriptome data demonstrated that soybean has differential expression of AP2 genes under cold stress. Additionally, during cold stress, most of these genes had the same patterns of expression but one gene (GLYMA_13G329700) is always upregulated irrespective of stress applied to soybean. This study revealed the characteristics of the AP2 gene family in soybean and provides valuable resources for further evolutionary and functional investigations of AP2 genes in this crop plant.

Keywords: *Glycine max* L., AP2 gene family, Characterization, Phylogenetic Analysis, Synteny analysis, Expression Analysis

INTRODUCTION

Cultivated soybean (*Glycine max* L.) is an economically important crop that belongs to the Fabaceae family. It is one of the world's most cultivated and versatile crops, valued for its high protein content and oil-rich seeds [1]. Soybean is utilized in various industries, including food production, animal feed, biofuels, and pharmaceuticals [2]. Gaining insight into the genetic composition of soybean is essential for enhancing crop productivity and quality as well as creating sustainable farming methods [3].

Soybean genome is complex and consists of approximately 20 chromosomes. It is a diploid species with a haploid genome size of about 1.1 billion base pairs. The genomic sequence of soybean was proposed firstly in 2010 and has been further studied afterward [4]. Investigation of these genomes has a great impact on the production and growth of soybeans. It excessively aided the scientists for further innovation on its composition, structure, gene location, gene region and functions [5]. A wide range of gene families found in soybeans support different defense mechanisms against biotic and abiotic stresses as well as plant development. An extensive range of resistance genes found in soybean are used in defense against environmental damage, diseases, and pests. Transcription factors are a class of regulatory proteins that command how other genes are expressed.

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Soybean has many transcription factor families that are associated with a range of processes related to development and stress responses, such as AP2/ERE, SBP, MYB, WRKY, NAC, and bZIP. Plant growth mechanisms are substantially regulated by the AP2 transcription factor family, also known as APETALA2 [6].

The transcriptome data generated by new technologies suggested the gene expression of AP2 genes is regulated by various environmental factors in soybean [7]. Different analyses on AP2 genes have greatly contributed to improve traits to boost the immunity and production of soybean in response to diverse stresses [8]. Soybean's huge genetic variation has captured the attention of scientists in plants biology and genetics field to explore different ways to improve this crop [9] [7]. The AP2 gene family holds great significance in understanding the genetic processes that underlie soybean improvement, yied potential and stress tolerance [10]. The AP2 genes are members of the AP2/EREBP (APETALA2/Ethylene Responsive Element Binding Protein) transcription factor super family, which is widely distributed in plants and have diverse functions in several metabolic and growth phases [11]. It is well recognized that AP2 transcription factors are essential for controlling the growth of flowers, germination of seeds, ripening of fruit, hormone signaling, and reactions to biotic and abiotic stresses. AP2 family is characterized by the AP2 domain that is the DNA conserved binding domain. The cysteine and histidine residues in this domain are arranged in a distinctive order to create structures resembling zinc fingers. The AP2 proteins can attach to DNA sequences and control the expression of target genes because of these zinc fingers [12]. Comprehending the molecular pathways governed by the AP2 genes can offer valuable perspectives on the management of the crucial developmental events in soybean [13]. The way soybean plants react to different environmental conditions has been linked to the AP2 genes as they are well known to play a role in regulating plant responses to cold, drought, and diseases [14, 15].

Knowing the molecular principles by which the AP2 gene contributes to stress responses can aid in the development of stress-tolerant soybean varieties with improved resilience to challenging environmental conditions [16]. Similarly, exogenous application of melatonin has led to alleviation of various stresses in different plants species and especially in soybean [17]. Additionally, application of melatonin leads to enhanced growth and development in the plants. The main objectives of this research article are to characterize AP2 gene family members for different attributes in soybean and their expression analysis under cold stress following the application of melatonin.

No study on the AP2 domain proteins in *G. max* has been reported, mostly because of the later release of the genome than in other species. Fortunately, with the release of the high-quality reference genome and annotation file downloaded from the Phytozome website (https://phytozome-next.jgi.doe.gov/), quick and efficient methods for understanding Soybean genetics and genomics have been developed rapidly. In this research article a total of 50 AP2 genes of the Glycine max genome, were firstly identified with a genome-wide scan on the latest genome and then a systematical analysis, including the gene phylogenetic relationship, protein-conserved domain, gene structure, chromosome localization, synteny analysis, conserved motif, and gene expression analyses were performed for these genes to investigate their evolutionary and functional features.

MATERIALS AND METHODS

Database Search and Sequence Retrieval

From the Plant TF Database, the soybean AP2 family member's DNA and protein sequences were retrieved. To validate the putative AP2 TFs, BLASTP on the NCBI webserver was employed. The detected AP2 TFs' gene accession numbers (GI), chromosomal counts, genomic data, and protein sizes were then obtained by searching the Phytozome database. The genomic nucleotide sequences for all the identified AP2 TFs were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The molecular weight and isoelectric point were calculated using the ExPASy Bioinformatics Resource Portal (http://web.expasy.org/compute_pi/) [18].

Conserved Domain Analysis, Intron/Exon Identification, and Chromosomal Mapping

The positions of the AP2 genes in *G. max* on the chromosomes were recorded using the NCBI database. Map Chart (v. 2.32) was used to create a chromosomal distribution map of the discovered non-redundant AP2 genes [19]. The gene structure, including the pattern of introns and exons, was visualized using the GSDS as described by [20]. The genomic DNA and CDS sequences of all the AP2 genes were submitted to the server for generating the gene structure maps, taking into consideration the intron phases. To get conserved domains within the soybean AP2 proteins, their protein sequences underwent analysis using MEME which was described by [21]. With the exception of limiting motif occurrence to 0 or 1 per sequence, setting the number of motifs at 50, defining the minimum number of sites for a motif as 5, and determining the ideal width of motifs to range from 10 to 55 residues, the analysis was carried out using the standard settings.

Phylogenetic Investigation of AP2 Proteins

The AP2 transcription factor (TF) protein sequences from soybean were used to construct a phylogenetic tree to determine the evolutionary relationship. Originally, 99 AP2 protein sequences were obtained from the Plant TFDB (https://planttfdb.gao-lab.org/) then 50 non-redundant protein sequences were selected for phylogenetic analysis. The multiple sequence alignment of these 50 non-redundant AP2 sequences was performed using Clustal Omega [22] at the following web address (https://www.ebi.ac.uk/Tools/msa/clustalo/). The resulting alignment was then utilized to generate a phylogenetic tree using the neighbor-joining method with 1000 bootstrap replicates. This was accomplished using the Molecular Evolutionary Genetics Analysis (MEGA v7.0) tool [23, 24].

Comparative Synteny Gene Analysis with *Arabidopsis thaliana*

For comparative synteny analysis, 30 protein sequences of AP2 gene family of *Arabidopsis thaliana* were obtained from plant PlantTF database server (https://planttfdb.gao-lab.org/). The 50 protein sequences of soyabean were uploaded in query FASTA format and *Arabidopsis thaliana* sequences were uploaded in database FASTA format on Circoletto webserver (http://tools.bat.infspire.org/circoletto/) by using the procedure given by [25].

***In silico* Gene Expression Analysis of AP2 Under Cold Stress**

Transcriptomic data of AP2 was obtained from NCBI. Sequence Read Archive (SRA) accession numbers were taken and then subjected to Galaxy Server to obtain Fragments per Kilobase of Transcript per Million mapped reads (FPKM) values of entire genome [26]. To investigate the expression of AP2 family genes in soybean in response to cold stress modulated by melatonin application the SRA data were used (SRA accession number PRJNA916852). Under bio project (PRJNA916852) total 18 runs were observed. FPKM values of understudy 48 genes of AP2 family were separated. Using these values Heatmap analysis was performed through TBtool [27].

RESULTS

Identification and Distribution of AP2 TFs Encoding Genes

Initially, 99 AP2 genes encoding putative AP2 TFs from soybean genome were identified. We then looked for major domains in the encoding proteins which were specific to AP2 in the retrieved genes. For this reason, the basic requirement for a gene to be included in the AP2 family was determined to be the presence of the entire AP2 domain. As a result, 49 AP2 genes were eliminated because their amino acid sequences' AP2 domains were comparable.

All these 50 genes are present on 19 chromosomes and no gene was found on the chromosome no. 20. The isoelectric point value had a range from 5.8161 to 10.1309, while the molecular weight ranged from 24.10 to 77.51 KDa, with an average of 50.80 KDa.

Genomic Localization and Mapping of AP2 Genes Using Map Chart

Insights into chromosomal positions and distribution chromosomal data were collected from SOYABASE webserver, and obtained values of chromosome positions on the chromosomes were arranged on excel sheet. This chromosomal data regarding the length of the chromosomes and positions of the gene were used for localization of genes on the corresponding chromosomes by using MapChart (Fig 1). According to the map chart, chromosome 1 contains 3 genes.

Similarly, chromosome 2 shows the presence of four genes. Chromosome 3 contains two genes Glyma.03G136100.1 and Glyma.03G177500.1. Chromosome 4 also has two genes. Likewise, Chromosome 5 contains two genes (Glyma.05G091200.1 is present in center of chromosome at scale 23, Glyma.05G108600.1 is present between middle and end of the chromosome at scale 29). The numbers and localization of the AP2 genes on soybean chromosomes is shown in Figure 1. Among them two genes are present on the scaffold.

Table 1: Detailed properties of identified AP2 Genes

TRANSCRIPTS	Location on Chrom	Start	End	Protein size	Domain Start	Domain End	PI	M Wt. (KDa)	Protein
Glyma.01G022500.1	Chr01	2246169	2251398	511	223	282	7.197	55861.4	XP_003517351.1
Glyma.01G188400.1	Chr01	52343828	52347518	503	149	198	8.1779	55637.5	XP_006573642.1
Glyma.01G195900.1	Chr01	52990264	52994010	616	274	333	7.5703	69425.4	XP_014631851.1
Glyma.02G087400.1	Chr02	7618899	7624414	417	161	210	8.2792	46347.2	XP_003520010.1
Glyma.02G179500.1	Chr02	30470574	30475871	393	113	170	8.2898	44062.2	XP_006575992.1
Glyma.02G185200.1	Chr02	32607457	32613210	448	90	149	9.0595	50165.1	XP_003519085.1
Glyma.02G207100.1	Chr02	39191302	39194205	366	48	107	6.7425	42175.7	XP_006575322.1
Glyma.03G136100.1	Chr03	35199502	35203392	424	61	120	9.0566	47086.6	XP_006576818.1
Glyma.03G177500.1	Chr03	39015668	39018941	460	124	173	6.7397	50271.7	XP_003520641.1
Glyma.04G047900.1	Chr04	3858051	3862348	664	297	356	7.8634	73455.8	XP_006578065.1
Glyma.04G154900.1	Chr04	35946733	35950623	348	44	100	7.2637	39632.7	XP_006579217.1
Glyma.05G091200.1	Chr05	21947028	21951107	534	178	227	6.4678	59057.7	XP_006579885.1
Glyma.05G108600.1	Chr05	28773212	28777586	666	319	378	6.5428	74367	XP_006579962.1
Glyma.06G049200.1	Chr06	3693118	3697685	661	297	356	7.7572	73129.3	XP_006581282.1
Glyma.06G225200.1	Chr06	30990649	30994490	352	44	100	7.2638	40076.3	XP_025984526.1
Glyma.07G021000.1	Chr07	1634020	1638370	291	30	89	6.6435	33105.4	XP_003529766.1
Glyma.07G038200.1	Chr07	3141188	3145227	580	246	305	6.8225	63750.9	XP_006583159.1
Glyma.07G199700.1	Chr07	36835549	36836275	218	2	44	10.1309	24107.3	XP_028242307.1
Glyma.08G220800.1	Chr08	17942538	17946419	314	50	109	8.1762	35882.6	XP_003530350.2
Glyma.08G227700.1	Chr08	18539711	18545318	427	55	114	6.4641	48188.1	NP_001357993.1
Glyma.08G279000.1	Chr08	37694278	37700595	448	163	222	8.2226	48905.1	XP_006585923.1
Glyma.08G297000.1	Chr08	41326370	41329183	408	72	131	9.7905	45816	XP_003530686.1
Glyma.09G199800.1	Chr09	42427981	42433197	513	222	281	7.1974	56063.4	XP_006587577.1
Glyma.09G240400.1	Chr09	46283701	46285756	393	71	130	7.879	44604.5	XP_006587765.1

Glyma.09G248200.1	Chr09	46951465	46956221	708	265	324	6.8158	77344.1	XP_028179766.1
Glyma.10G116600.1	Chr10	29253588	29259287	414	156	205	9.1957	45539.2	XP_003535179.1
Glyma.10G171400.1	Chr10	40494362	40498737	576	209	265	6.7662	63705.6	XP_014618314.1
Glyma.11G045800.1	Chr11	3414233	3418061	613	276	335	7.4276	68684.6	XP_006590617.1
Glyma.11G053800.1	Chr11	4048454	4052090	497	236	286	8.0285	54211.6	XP_028190290.1
Glyma.11G131900.1	Chr11	10107578	10113569	599	209	263	6.6603	66535.2	XP_006590237.1
Glyma.12G056300.1	Chr12	4099866	4103287	554	163	222	6.6209	61370.2	XP_006592171.1
Glyma.12G073300.1	Chr12	5425771	5430181	494	156	205	7.5601	54101.8	XP_006592242.1
Glyma.13G096900.1	Chr13	21189580	21193427	529	146	205	7.9813	56967.3	XP_003541472.1
Glyma.13G329700.1	Chr13	42422212	42426795	477	136	185	7.6544	52685.7	XP_003542008.1
Glyma.14G089200.1	Chr14	8095483	8099513	600	227	286	7.3108	65992.8	XP_014622036.1
Glyma.15G044400.1	Chr15	3527818	3532707	486	136	185	8.4502	53752	XP_003547042.2
Glyma.15G221600.1	Chr15	40067762	40073180	413	53	112	5.8161	46503.3	XP_025981368.1
Glyma.16G007400.1	Chr16	605286	609287	575	244	303	6.4278	63424.4	XP_006598829.1
Glyma.17G062600.1	Chr17	4812929	4816734	578	299	350	7.9154	62166.2	XP_028211618.1
Glyma.17G070800.1	Chr17	5546049	5549115	371	49	108	6.6808	42767.3	XP_003550676.1
Glyma.17G158300.1	Chr17	13595432	13600012	685	326	385	6.8004	76548.3	XP_014625550.1
Glyma.17G170300.1	Chr17	15975183	15979979	533	176	225	6.5132	58862.6	XP_006600938.1
Glyma.18G125200.1	Chr18	16381350	16384071	407	72	131	9.5701	45717.9	XP_003553203.1
Glyma.18G148000.1	Chr18	25334271	25341458	440	159	218	8.6274	48377.9	XP_003551311.1
Glyma.18G244600.1	Chr18	53236144	53240803	710	369	420	6.6383	77512.6	XP_003552462.1
Glyma.18G256000.1	Chr18	54212716	54215640	393	71	130	6.8086	44614.6	XP_006602898.1
Glyma.19G138000.1	Chr19	39934457	39938605	419	60	119	9.0566	46518.1	XP_003554171.1
Glyma.19G178200.1	Chr19	43733666	43738337	495	159	208	8.0195	54330.3	XP_003554357.2
Glyma.U022100.1	scaffold_21	3550791	3555149	449	160	209	6.191	49127	XP_028189749.1
Glyma.U037700.1	scaffold_44	4329	9453	500			7.3033	55890.6	XP_025983430.1

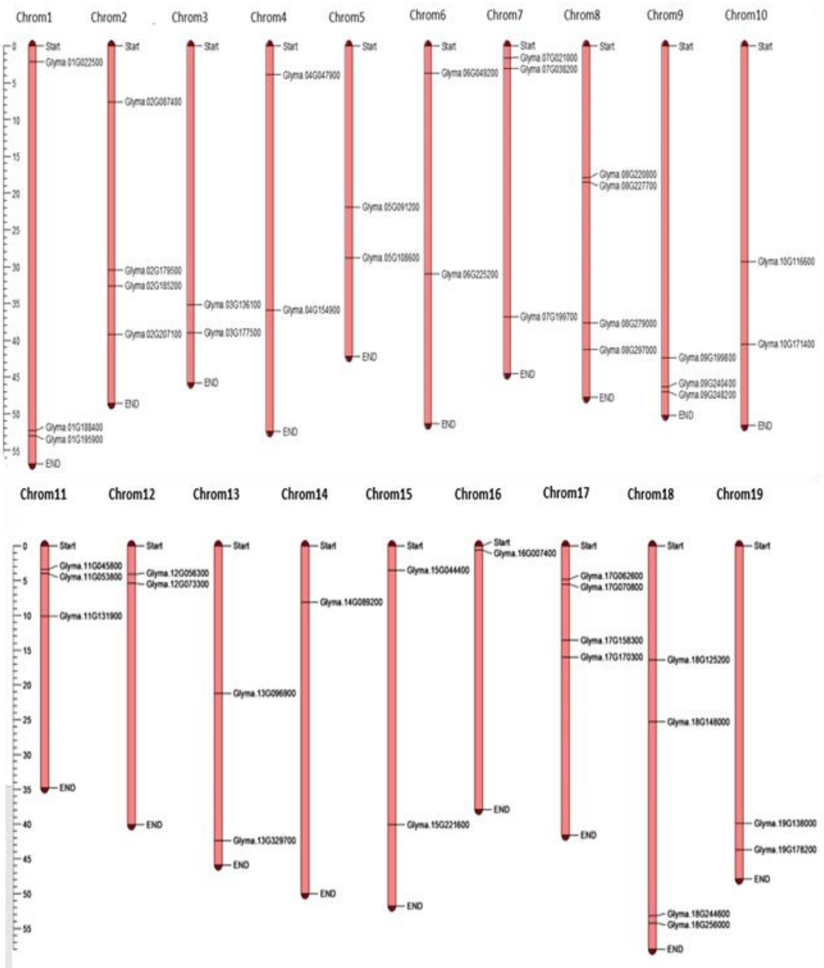

Figure 1. The positioning of 48 AP2 genes across soybean chromosomes is depicted in the figure. Each bar at the top corresponds to a specific chromosome (1–19) in soybean. The left side of the figure indicates the scale in Mb. On the right side of each chromosome, the location of each AP2 gene is marked.

Unveiling the AP2 Gene Family Classification, Gene Structure, and Conserved Domain Investigations

The gene structure i.e., the intron/exon distribution pattern of 50 AP2 genes was also determined to gain further insights into the evolution of AP2 gene family in soybean. The position and intron–exon distribution pattern in the genomic region of a gene is used as supporting evidence of expansion pattern of a gene family and its evolutionary relationship with its ancestors.

Soybean AP2 genes exhibited a diversity with respect to the number of introns e.g., ranging from 2 to 10. Despite the differences in the size of their genomic regions, a somewhat conserved gene structure within the groups of phylogenetic trees has been observed (Fig 2) except Glyma.11G131900. The gene structure of Glyma.08G225200 was observed because of having maximum portion of exons and only two introns, while there is absence of non-coding region due to very short size of its genomic DNA sequence i.e., 0.7 Kb (Fig 2). One gene (Glyma.U037700) was found to have maximum portion of introns i.e., 10 introns, 11 exons and 2 untranslated regions. The gene Glyma.18G148000 is observed to have large size as compared to other genes i.e., 7.2 Kb (Fig 2).

The identification of conserved domains in a gene family also serves as supporting evidence to verify the gene duplication events during evolution; and to dissect their functional conservation. For this purpose, the protein sequences of all the AP2 TFs were subjected to MEME, for the identification of conserved domains. All the genes show duplication in conserved domains which is observed by NCBI. The conserved domain was shown with grey color in Fig. 3 while colored portion shows the non-conserved region Among them, domains 1 and 2 represent the characteristic AP2- DNA binding domain which is absolutely conserved among all the 50 AP2 protein sequences.

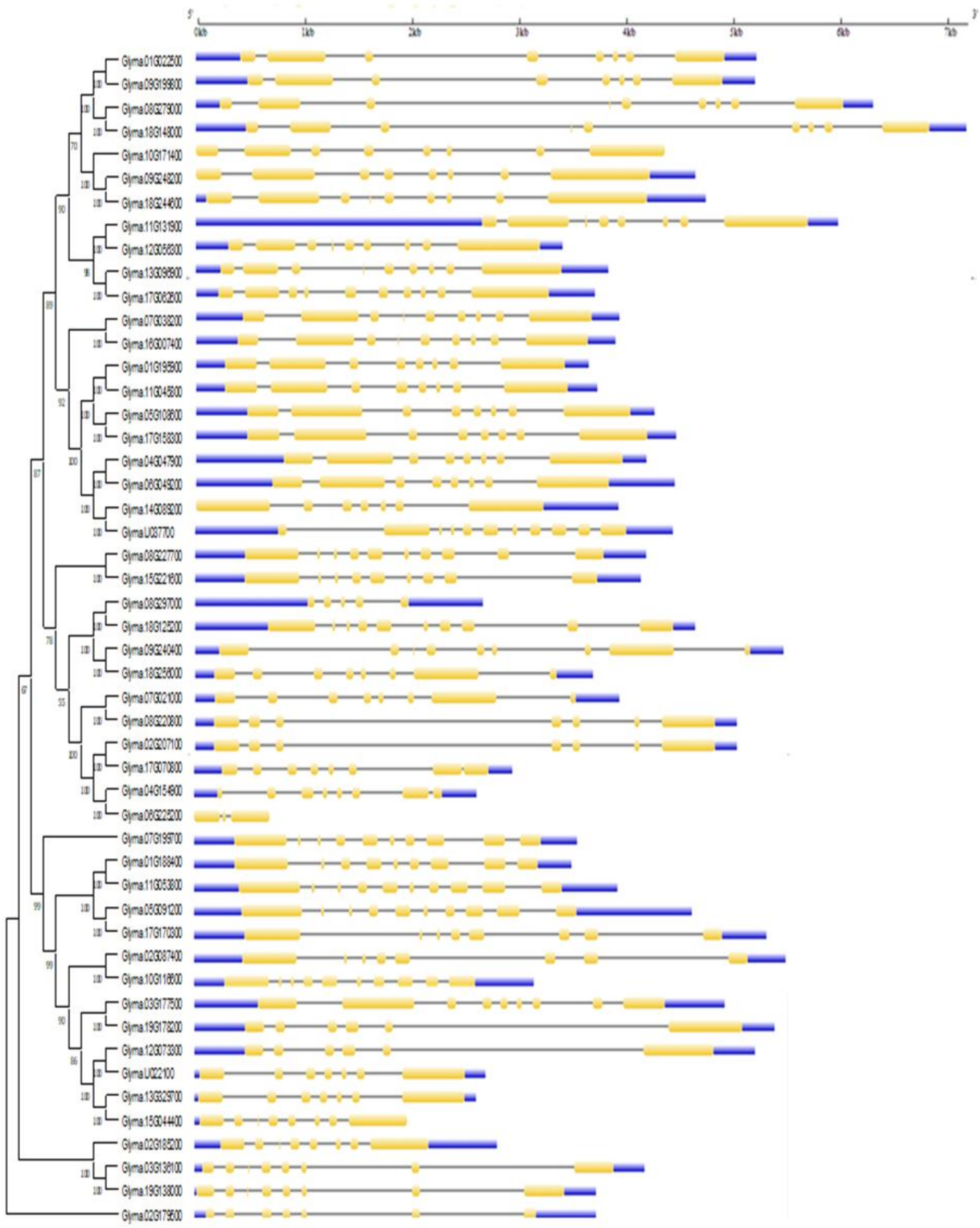

Figure 2. Phylogenetic tree-based clustering and structure of AP2 TFs. An unrooted phylogenetic tree was created based on the full-length peptide sequences of AP2 with 1000 bootstraps. Using various hues, classification is displayed at the base of the phylogenetic tree. An analysis of the exon-intron structure in AP2 genes. Exons are represented by yellow boxes and introns by a grey line. The untranslated region (UTR) is indicated by the blue boxes. Gray lines represent the non-conserved sequences.

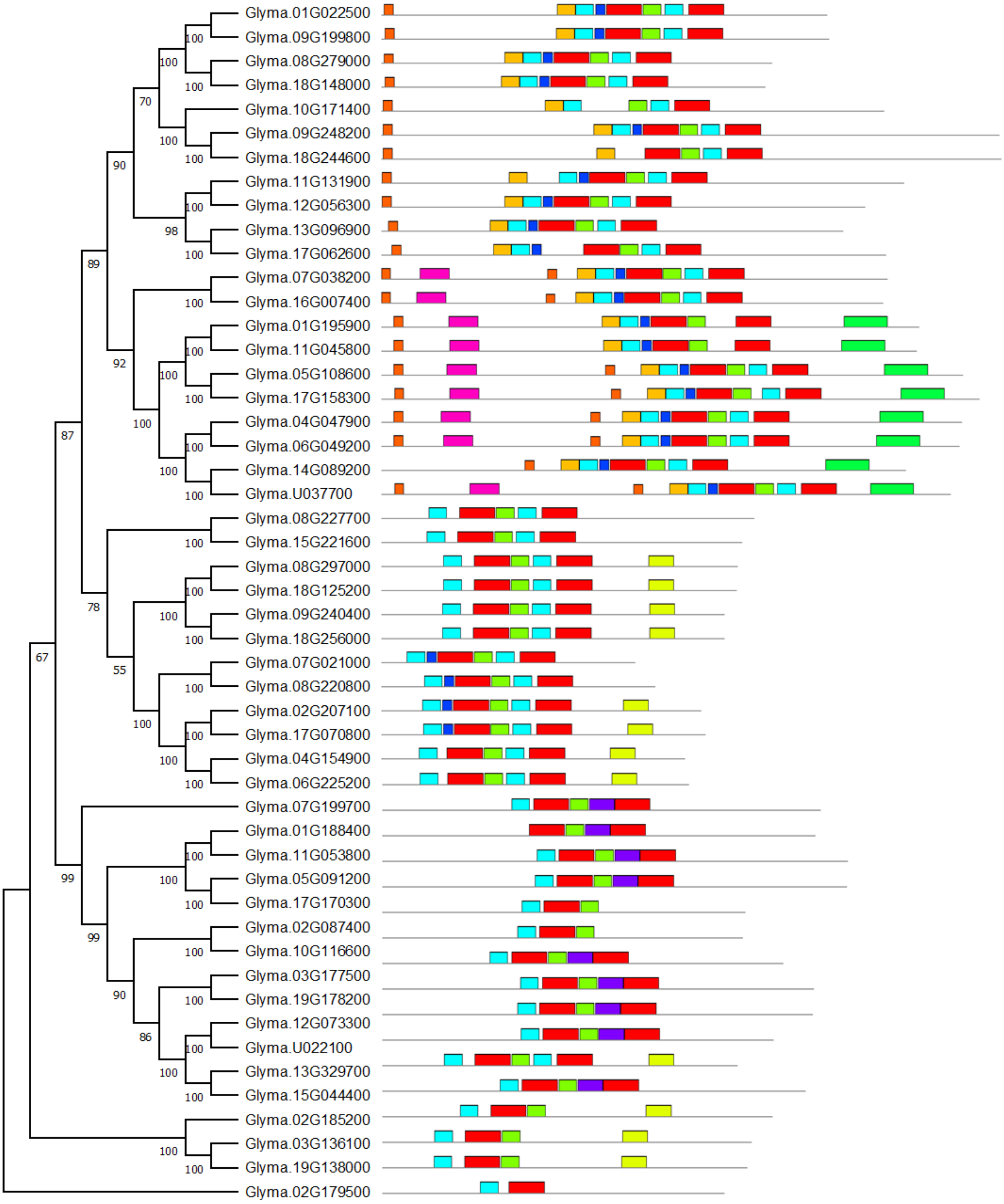

Figure 3. Motif analysis is done by MEME and conserved regions are observed which are shown in above diagram.

AP2 Gene Correlation between *Arabidopsis thaliana* & *Glycine max* L. through Synteny Analysis

A comparative study of the protein sequences from model plant, *Arabidopsis thaliana* and soybean was done by using Circoletto tool which provides information about the evolutionary relationship and conservation of AP2 genes presented in both species (Fig 4). After running protein sequence of both species, each gene shows evolutionary relation with one another. Genes of same color are connected, which shows there is evolutionary relation between genes. Query sequence is magnified as a bulging region in the histogram as compared the subject sequence. (Fig 4) shows syntenic resemblance among *Glycine max* L. and *Arabidopsis thaliana*. All labels are clockwise in direction. Through this analysis we identified AT3G20840.1 and AT1G51190.1 genes showed syntenic relationship with Glyma.11G131900 and Glyma.12G056300. other two genes Glyma.09G199800 and Glyma.01G022500 expressing syntenic behavior with AT5G10510.3, AT5G10510.2 and AT5G10510.1 three genes of Arabidopsis.

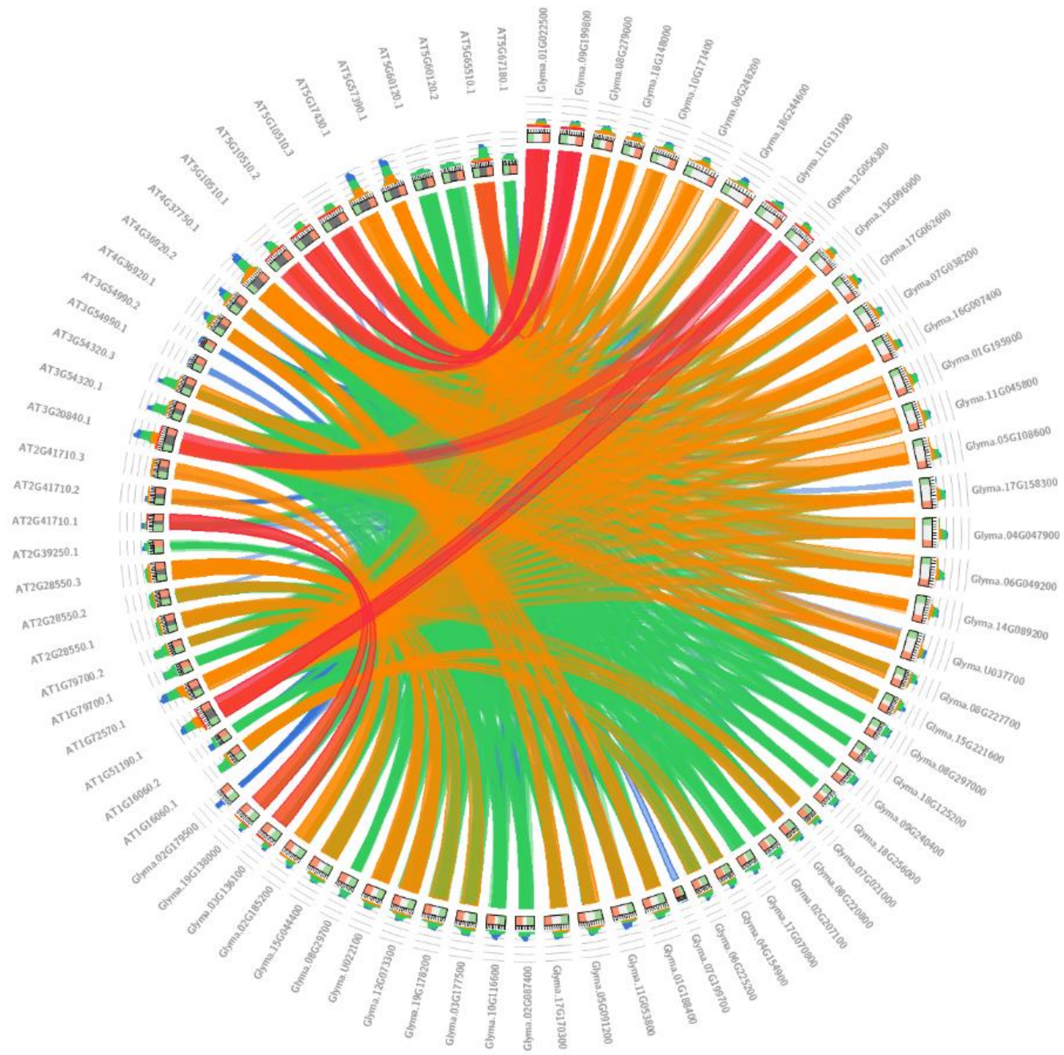

Figure 4. Synteny analysis between *Arabidopsis thaliana* and *Glycine max* L. genes which shows evolutionary relation of both species in AP2 gene family. In this analysis blue, green, orange, and red colors show 50, 75, 99.9 and 100% identity respectively.

Expression analysis of AP2 genes under various cold stress stages

A transcriptome wide study using SRA database from NCBI made us able to identify the expression of 48 AP2 genes in different treatments under cold stress (Figure 5). Under control and melatonin application at room temperature treatment first three genes (GLYMA_08G220800, GLYMA _07G021000, GLYMA_10G116600) show slightly differetial expression against cold and under application of malatonin. Only one gene, GLYMA_13G329700 was identified with the highest expression under control and all five treatments. At the last one gene (GLYMA_12G073300) gives very high expression under low temperature treatment after 24 hours treatment and after 24 hours melatonin application under low temperature treatment. Further heat-map profile shows that other six genes which is (GLYMA_02G087200, GLYMA_15G044400, GLYMA_05G091200, GLYMA_17G170300, GLYMA_01G188400, GLYMA_11G053800) express moderately in all six treatments under cold stress. Overall results demonstrated no differential gene regulation of AP2 genes by application of melatonin.

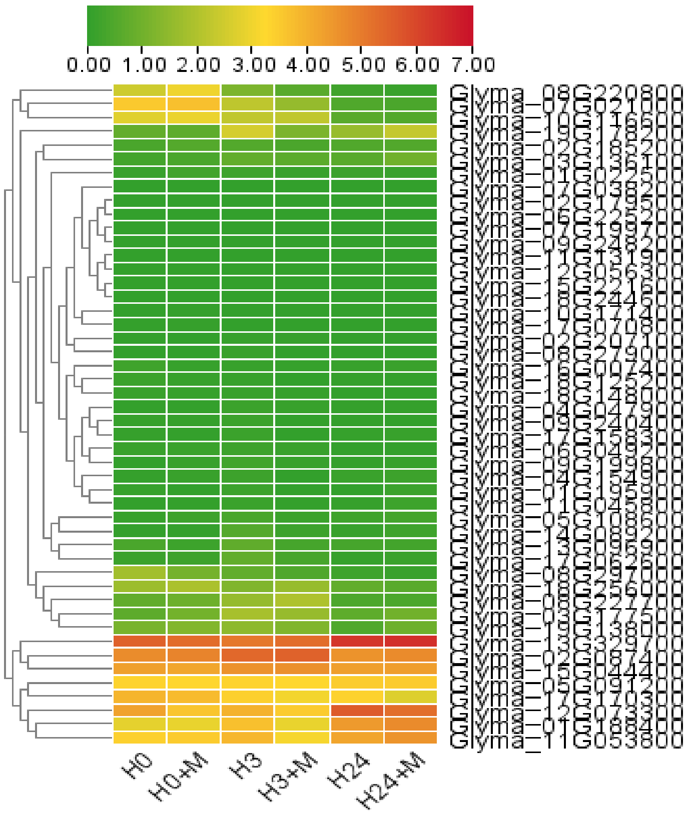

Figure 5. Heatmap chart of 48 AP2 genes of Glycine max L. for expression of genes under cold stress. Where (H) represents the number of hours of cold stress while (M) represents the melatonin sprayed to the plants. Maximum expression is shown by red color whereas the minimum expression was shown by boxes in green color. The color scale for expression is given in the legend.

DISCUSSION

The AP2 (APETALA2) gene family plays a critical role in various aspects of plant development, encompassing flower development, seed maturation and responses to environmental stresses [28]. In this investigation, we conducted a comprehensive study to uncover and characterize AP2 genes in the soybean genome.

In the present study, 50 non redundant AP2 genes were identified and characterized. Comparison of this number with other species showed that AP2 genes in soyabean is greater than that in Tiepishihu (14 AP2) [29], Arabidopsis (18 AP2) [30], peach (21)[31], sweet potato (21 AP2) [32], rice (26 AP2) [33] and maize (44 AP2) [34]. While less than that in wheat (62 AP2) [35] and rapeseed (58 AP2) [36]. This comparison suggests that the number of genes expressing AP2 proteins is not correlated with the size of the corresponding plant species' genomes.

By unraveling the genomic organization, gene structures, conserved domains, and expression patterns of AP2 genes, we acquire valuable insights into their functional roles in soybean development and stress responses [37]. This knowledge contributes to our understanding of the regulatory mechanisms underlying plant growth and adaptation, which may hold significant implications for crop improvement and agricultural practices. Comparative analysis of AP2 genes across different plant species reveals both conserved and divergent features in terms of gene structure, expression patterns, and functional roles. The classification of soybeans using phylogenetic trees followed a similar pattern to other plant species. The analysis of AP2s regarding the distribution of introns and exons and conserved domains revealed that members of the identical group in the evolutionary tree share both gene structure and domain conservation. Gene structure analysis involved scrutinizing the exon-intron organization of AP2 genes, thereby providing insights into their structural diversity like number of introns ranges from 2-10 like in Mosa bamboo [38]. Higher number of introns were also reported like 5-14 in sweet potato [32] and 5-114 in Japonica rice [11].

In this study, to find orthologs of AP2 genes between *G. max* and *A. thaliana*, a comparative synteny study was conducted. According to the findings, 30 Arabidopsis genes and 50 *G. max* genes exhibited high similarity (identity > 70%). Through this analysis we identified AT3G20840.1and AT1G51190.1 genes showed orthologous relationship with Glyma.11G131900 and Glyma.12G056300. Prior research has revealed that orthologous gene pairs 54 and 84 showed 100% identity between BnAP2/ERF genes with *B. rapa* and *B. oleracea* genes, respectively, no *A. thaliana* gene was found to exhibit 100% identity with BnAP2 genes [36].

Plant genetic improvement relies on the identification and functional verification of TFs, which can yield valuable candidate gene resources. According to earlier studies, AP2 TFs are important regulators of certain stress responses and developmental processes, making them promising candidates for plant betterment [39]. Furthermore, we explored the functional roles of AP2 genes by examining their expression patterns under various stress conditions at different timepoints. Leveraging publicly available transcriptomic data, already investigated the expression profiles of AP2 genes in diverse organs [40]. Additionally, we scrutinized their expression responses to cold modulated with melatonin.

The expression of AP2 genes was investigated in this work under cold stress followed by melatonin application. At the seedling stage, soybeans were exposed to cold stress and were sprayed with melatonin at 0H, 3H, and 24H. Under cold stress, one gene, GLYMA_13G329700, exhibits up-regulation. Under varied melatonin stress treatments, six other genes also displayed upregulation. Previously, AP2 genes, OsAP2-4.1, OsAP2-6.1, OsAP2-5.3, OsAP2-6.4, OsAP2-6.2, and OsAP2-8.1 were up-regulated in under heat stress in rice, showed their importance in heat tolerance [33].

Cotton AP2 genes have been characterized for their roles in fiber development, including fiber initiation, elongation, and secondary cell wall biosynthesis [41]. Gene expression analysis primarily focuses on how genes are activated (expressed) or inactivated in diverse cells and environmental contexts. The proteins that are expressed because of this process ultimately affect the characteristics and functions of the plants in response to stresses [42-43].

CONCLUSIONS

In summary, this study discovered 50 AP2 encoding genes in soybean, which is an important oil seed crop. The research involved classifying and characterizing these genes based on their gene structure and conserved domains. Comparative phylogenetic analyses indicated that AP2 classes are clustered on the basis of similar motifs and intron-exon patterns in soybean. Most of the AP2 genes were not differentially expressed under normal, cold and cold+melatonin applications. GLYMA_13G329700 gene showed maximum expression under all treatments. This approach will help for the functional characterization of AP2 TFs much faster. Over all these analyses will help to increase the functional characterization of AP2 TFs especially in response to biotic and abiotic stress.

REFERENCES

1. Macharia, J. M., Mwangi, R. W., Rozmann, N., Zsolt, K., Varjas, T., Uchechukwu, P. O., ... & Raposa, B. L. 2022. Medicinal plants with anti-colorectal cancer bioactive compounds: Potential game-changers in colorectal cancer management. Biomedicine & Pharmacotherapy, 153, 113383.

2. Singh, P., & Krishnaswamy, K. 2022. Sustainable zero-waste processing system for soybeans and soy by-product valorization. Trends in Food Science & Technology, 128, 331-344.

3. Meena, R. S., Vijayakumar, V., Yadav, G. S., & Mitran, T. 2018. Response and interaction of Bradyrhizobium japonicum and arbuscular mycorrhizal fungi in the soybean rhizosphere. Plant Growth Regulation, 84, 207-223.

4. Varala, K. K. 2011. Genome composition of Glycine max and sequence diversity among cultivated and exotic accessions. University of Illinois at Urbana-Champaign.

5. Stacey, G., Vodkin, L., Parrott, W. A., & Shoemaker, R. C. 2004. National Science Foundation-sponsored workshop report. Draft plan for soybean genomics.

6. Zeng, D., Silva, J. A. T. D., Zhang, M., Yu, Z., Si, C., Zhao, C., ... & Duan, J. 2021. Genome-wide identification and analysis of the APETALA2 (AP2) transcription factor in Dendrobium officinale. International Journal of Molecular Sciences, 22(10), 5221.

7. Severin, A. J., Woody, J. L., Bolon, Y. T., Joseph, B., Diers, B. W., Farmer, A. D., ... & Shoemaker, R. C. 2010. RNA-Seq Atlas of Glycine max: a guide to the soybean transcriptome. BMC plant biology, 10, 1-16.

8. Pazhamala, L. T., Kudapa, H., Weckwerth, W., Millar, A. H., & Varshney, R. K. 2021. Systems biology for crop improvement. The plant genome, 14(2), e20098.

9. Nawaz, M. A., Lin, X., Chan, T. F., Lam, H. M., Baloch, F. S., Ali, M. A., ... & Chung, G. 2021. Genetic architecture of wild soybean (Glycine soja Sieb. and Zucc.) populations originating from different East Asian regions. Genetic Resources and Crop Evolution, 68, 1577-1588.

10. Dubey, A., Kumar, A., Abd_Allah, E. F., Hashem, A., & Khan, M. L. 2019. Growing more with less: breeding and developing drought resilient soybean to improve food security. Ecological Indicators, 105, 425-437..

11. Sharoni, A. M., Nuruzzaman, M., Satoh, K., Shimizu, T., Kondoh, H., Sasaya, T., ... & Kikuchi, S.

2011. Gene structures, classification and expression models of the AP2/EREBP transcription factor family in rice. Plant and cell physiology, 52(2), 344-360.
12. Agarwal, P., Reddy, M. P., & Chikara, J. 2011. WRKY: its structure, evolutionary relationship, DNA-binding selectivity, role in stress tolerance and development of plants. Molecular biology reports, 38, 3883-3896.
13. Yao, S., Lan, Z., Huang, R., Tan, Y., Huang, D., Gu, J., & Pan, C. 2021. Hormonal and transcriptional analyses provides new insights into the molecular mechanisms underlying root thickening and isoflavonoid biosynthesis in Callerya speciosa (Champ. ex Benth.) Schot. Scientific Reports, 11(1), 9.
14. Qiu, Y., & Yu, D. 2009. Over-expression of the stress-induced OsWRKY45 enhances disease resistance and drought tolerance in Arabidopsis. Environmental and experimental botany, 65(1), 35-47.
15. Ali, M. A., Abbas, A., Kreil, D. P., & Bohlmann, H. 2013. Overexpression of the transcription factor RAP2. 6 leads to enhanced callose deposition in syncytia and enhanced resistance against the beet cyst nematode Heterodera schachtii in Arabidopsis roots. BMC plant biology, 13, 1-17.
16. Jha, U. C., Bohra, A., Jha, R., & Parida, S. K. 2019. Salinity stress response and ‘omics’ approaches for improving salinity stress tolerance in major grain legumes. Plant cell reports, 38, 255-277.
17. Qari, S. H., Hassan, M. U., Chattha, M. U., Mahmood, A., Naqve, M., Nawaz, M., ... & Aljabri, M. 2022. Melatonin induced cold tolerance in plants: physiological and molecular responses. Frontiers in Plant Science, 13, 843071.
18. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S. E., Wilkins, M. R., Appel, R. D., & Bairoch, A. 2005. Protein identification and analysis tools on the ExPASy server (pp. 571-607). Humana press.
19. Voorrips, R. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. Journal of heredity, 93(1), 77-78.
20. Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., & Gao, G. 2015. GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics, 31(8), 1296-1297.
21. Bailey, T. L., Johnson, J., Grant, C. E., & Noble, W. S. 2015. The MEME suite. Nucleic acids research, 43(W1), W39-W49.
22. Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., ... & Higgins, D. G. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular systems biology, 7(1), 539.
23. 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.
24. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, 35(6), 1547.
25. Darzentas, N. 2010. Circoletto: visualizing sequence similarity with Circos. Bioinformatics, 26(20).
26. Afgan, E., Baker, D., Batut, B., Van Den Beek, M., Bouvier, D., Čech, M., ... & Blankenberg, D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic acids research, 46(W1), W537-W544.
27. 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.
28. Licausi, F., Giorgi, F. M., Zenoni, S., Osti, F., Pezzotti, M., & Perata, P. 2010. Genomic and transcriptomic analysis of the AP2/ERF superfamily in Vitis vinifera. BMC genomics, 11, 1-16.
29. Zeng, D., Silva, J. A. T. D., Zhang, M., Yu, Z., Si, C., Zhao, C., ... & Duan, J. 2021. Genome-wide identification and analysis of the APETALA2 (AP2) transcription factor in Dendrobium officinale. International Journal of Molecular Sciences, 22(10), 5221.
30. Nakano, T., Suzuki, K., Fujimura, T., & Shinshi, H. 2006. Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant physiology, 140(2), 411-432.
31. Zhang, C. H., Shangguan, L. F., Ma, R. J., Sun, X., Tao, R., Guo, L., ... & Yu, M. L. 2012. Genome-wide analysis of the AP2/ERF superfamily in peach (Prunus persica). Genet Mol Res, 11(4), 4789-4809.
32. He, S., Hao, X., He, S., Hao, X., Zhang, P., & Chen, X. 2021. Genome-wide identification, phylogeny and expression analysis of AP2/ERF transcription factors family in sweet potato. BMC genomics, 22(1), 748.
33. Ahmed, S., Rashid, M. A. R., Zafar, S. A., Azhar, M. T., Waqas, M., Uzair, M., ... & Atif, R. M. 2021. Genome-wide investigation and expression analysis of APETALA-2 transcription factor subfamily reveals its evolution, expansion and regulatory role in abiotic stress responses in Indica Rice (Oryza sativa L. ssp. indica). Genomics, 113(1), 1029-1043.
34. Zhang, J., Liao, J., Ling, Q., Xi, Y., & Qian, Y. 2022. Genome-wide identification and expression profiling analysis of maize AP2/ERF superfamily genes reveal essential roles in abiotic stress tolerance. BMC genomics, 23(1), 125.

35. Zhao, Y., Ma, R., Xu, D., Bi, H., Xia, Z., & Peng, H. 2019. Genome-wide identification and analysis of the AP2 transcription factor gene family in wheat (Triticum aestivum L.). Frontiers in plant science, 10, 1286.
36. Ghorbani, R., Zakipour, Z., Alemzadeh, A., & Razi, H. 2020. Genome-wide analysis of AP2/ERF transcription factors family in Brassica napus. Physiology and molecular biology of plants, 26, 1463-1476.
37. Liu, C., & Zhang, T. 2017. Expansion and stress responses of the AP2/EREBP superfamily in cotton. BMC genomics, 18, 1-16.
38. Wu, H., Lv, H., Li, L., Liu, J., Mu, S., Li, X., & Gao, J. 2015. Genome-wide analysis of the AP2/ERF transcription factors family and the expression patterns of DREB genes in Moso Bamboo (Phyllostachys edulis). Plos one, 10(5), e0126657.
39. Kabir, S. M. T., Hossain, M. S., Bashar, K. K., Honi, U., Ahmed, B., Emdad, E. M., ... & Islam, M. S. 2021. Genome-wide identification and expression profiling of AP2/ERF superfamily genes under stress conditions in dark jute (Corchorus olitorius L.). Industrial Crops and Products, 166, 113469.
40. Xie, Z., Nolan, T. M., Jiang, H., & Yin, Y. 2019. AP2/ERF transcription factor regulatory networks in hormone and abiotic stress responses in Arabidopsis. Frontiers in plant science, 10, 437723.
41. Michailidis, G., Argiriou, A., Darzentas, N., & Tsaftaris, A. 2009. Analysis of xyloglucan endotransglycosylase/hydrolase (XTH) genes from allotetraploid (Gossypium hirsutum) cotton and its diploid progenitors expressed during fiber elongation. Journal of plant physiology, 166(4), 403-416.
42. Rutter, M., Moffitt, T. E., & Caspi, A. 2006. Gene–environment interplay and psychopathology: Multiple varieties but real effects. Journal of child Psychology and Psychiatry, 47(3-4), 226-261.
43. Berrar, D. P., Dubitzky, W., & Granzow, M. (Eds.). 2003. A practical approach to microarray data analysis (pp. 15-19). New York: Kluwer academic publishers.