The Pharma Innovation Journal 2023; 12(12): 219-227

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 219-227

www.thepharmajournal.com
Received: 24-09-2023
Accepted: 28-10-2023

Alamgir
Research Scholar, Department of Agricultural Biotechnology, S. VPUA& T. Meerut, Uttar Pradesh, India

Pushpendra Kumar
Professor, Department of Agricultural Biotechnology, S. VPUA& T. Meerut, Uttar Pradesh, India

Mukesh Kumar
Professor, Department of Agricultural Biotechnology, S. VPUA& T. Meerut, Uttar Pradesh, India

Vaishali
Professor, Department of Agricultural Biotechnology, S. VPUA& T. Meerut, Uttar Pradesh, India

MK Yadav
Professor, Department of Agricultural Biotechnology, S. VPUA& T. Meerut, Uttar Pradesh, India

Mukesh Kumar
Professor, Department of Genetics and Plant Breeding, S. VPUA& T. Meerut, Uttar Pradesh, India

Corresponding Author:
Pushpendra Kumar
Professor, Department of Agricultural Biotechnology, S. VPUA& T. Meerut, Uttar Pradesh, India

Molecular characterization and expression analysis of PhFBP20 MADS box gene in *Petunia hybrida*

Alamgir, Pushpendra Kumar, Mukesh Kumar, Vaishali, MK Yadav and Mukesh Kumar

Abstract
The *Petunia hybrida*, an ornamental plant is a member of the Solanaceae family. MADS box transcription factors (TFs) are DNA binding proteins, essential for several plant developmental processes, including the determination of floral organs. In this study, the open reading frame of PhFBP20 gene consists of 651bp nucleotides encoded 216 amino acids which is isolated, cloned and characterized. Phylogenetic analysis showed that PhFBP20 is similar to AGL20 of *A. thaliana* and *SOC1* of *S. lycopersicum*. The expression analysis revealed that PhFBP20 preferentially expressed in initial bud and leaf at higher rate in comparison to mature bud and open flower which suggest its involvement during floral transition pathways. Further, sequence analysis, identifies variations in the nucleotide (7) and amino acid (4) sequences within the MADS Box domain of the isolated PhFBP20 gene from *Petunia hybrida* and the FBP20 gene of *Petunia axillaris* obtained from the NCBI database. No change in nucleotide and amino acid sequence of isolated PhFBP20 from *Petunia hybrida* and FBP20 of *Petunia hybrida* gene obtained from NCBI database. These results expand our knowledge of flower formation and aid in the continued research of the MADS-Box gene family in many plant species.

Keywords: *Petunia hybrida*, MADS box, PhFBP20, motifs, expression analysis

Introduction
The bedding plant *Petunia hybrida* is a member of the Solanaceae family, which contains several agronomically significant species that are used as food, drugs and ornamental (Knapp *et al*., 2004) [7]. It is a widely used model system for investigating the evolution of floral organs and flavonoids production (Vandenbussche *et al*., 2016) [28]. Transcription factors (TFs) are regulatory proteins responsible for modulating the transcriptional rates of their target genes by interacting with DNA-binding domains (DBDs) that enable them to recognize specific DNA sequences which located in promoter regions where, they activate or deactivate the upstream signaling cascades. (Inukai *et al*., 2017) [13]. TFs play important roles in all biological function, including development, growth and responses to environmental stimuli. Furthermore, they are believed to play significant roles in the evolutionary processes of species (Riano *et al*., 2007) [22]. MADS-domain Tfs are essential components of regulatory networks that govern various developmental pathways across plants, animals, and fungi (Garay-Arroyo *et al*., 2013; Cao *et al*., 2016; Thangavel and Nayar, 2018) [8, 5, 26]. The acronym "MADS" is derived from the first four MADS-domain proteins discovered: M for Minichromosome Maintenance factor 1 from Saccharomyces cerevisiae, A for AGAMOUS (AG) from *Arabidopsis thaliana*, D for DEFICIENS from Antirrhinum majus, and S for Serum Response Factor (SRF) from Homo sapiens (Norman *et al*., 1988; Passmore *et al*., 1988; Jarvis *et al*., 1989; Sommer *et al*., 1990; Yanofsky *et al*., 1990) [19, 21, 14, 25, 29].
MADS-domain proteins have been extensively studied across different organisms and are known to participate in various developmental processes in plants (Smaczniak *et al*., 2012a) [24]. Gene duplication occurred before to the divergence of plants and animals, resulting in the emergence of two distinct lineages of MADS-domain proteins. These lineages are distinguishable by the highly conserved MADS box and other domains: Type I or SRF-like genes and Type II or MEF2-like genes (Myocyte Enhancer Factor 2) (Becker and Theißen, 2003; Alvarez-Buylla *et al*., 2000) [4, 1]. The Type II lineage, also termed MIKC-type in plants, subdivided into MICKc and MICK* groups. Furthermore, on the basis of phylogenetic studies, MIKCc contains 13 different gene subfamilies or clades (Parenicová *et al*., 2003; Becker and Theißen, 2003) [20, 4]. As land plants evolved, the number and functional diversity of increased due to multiple gene and genome duplications of MADS-box genes.

For instance, Arabidopsis has 108 members of MADS-box genes (Fan *et al*., 2013; Kaufmann *et al*., 2005) [7, 15].
In present study, the isolation of *Petunia hybrida* PhFBP20 MADS box gene was workout to assess the role of this gene with molecular perspective in flowering. Our study contributes a better understanding of MIKC- type MADS box genes PhFBP20 and function using *Petunia hybrida*. Here, we report the isolation and expression analysis of PhFBP20 MADS box gene from different tissue and flower stages of *Petunia hybrida*.

Materials and Methods

Plant Materials and Growth Conditions

Petunia hybrida Seeds were collected from Department of Agricultural biotechnology of SVPUA&T, Meerut. Plants were grown from these seeds at 20- 24 °C were maintained in the field laboratory of Department of Agricultural Biotechnology of SVPUA&T Meerut. RNA was isolated from initial bud, mature bud, open flower, sepal, petal and leaf of *Petunia hybrida* shown in Fig 1.

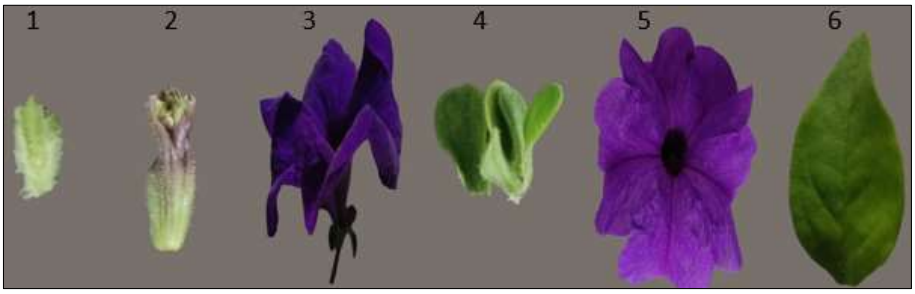

Fig 1: Different developmental stage of *Petunia hybrida* flower used for RNA isolation: 1) Initial bud, 2) Mature bud, 3) Open flower, 4) Sepal, 5) Petal, 6) Leaf

Cloning and Sequencing of PhFBP20 Gene

RNA was isolated from various stages and tissue of flower from *Petunia hybrida* using the some modification of standard protocol by Trizol method and cDNA was synthesized using reverse transcriptase (GeNei, Bangalore). The gene specific primers were designed using primer3 (https://bioinfo.ut.ee/primer3-0.4.0/). The PhFBP20 gene was PCR amplified from leaf cDNA using gene-specific primers (FBP20-*F*: 5′-ATGGTGAGAGGAAAAACTC -3′; FBP20-*R:* 5′CACCAATTAATTCTGTAAGCG - 3′) by initial denaturation for 3 minutes at 94 °C, denaturation for 1 minute at 94 °C, annealing for 55 seconds at 49 °C, extension for 1 minute at 72 °C and final extension for 10 minutes at 72°C. This PCR process was repeated for 35 cycles. The resulting amplified products of PhFBP20 were 651 base pairs in length. To further analyze and study the PhFBP20 gene, it was cloned into a pGEM-T easy vector (Promega) and confirmed through sequencing. The sequencing process was conducted at the DNA sequencing facility of Centyle Biotech Private Limited. Subsequently, the sequence was trimmed and assembled using BioEdit software to ensure its accuracy and to retain only the coding regions of the gene. The obtained sequencing data was validated using BLAST analysis (http://www.ncbi.nlm.nih.gov/blast/). The nucleotide sequence of PhFBP20 was used for nucleotide composition by using BioEdit software. The nucleotide sequence was then used to predict the amino acid sequence and its amino acid composition was analysed by BioEdit software (Hall *et al*., 2011) [12].

Sequence Alignment and the Construction of Phylogenetic Tree

The nucleotide sequence of the *Petunia hybrida* PhFBP20 MADS Box gene was subjected to multiple sequence alignment with the amino acid sequences of other MIKC-type MADS-Box genes from *Arabidopsis thaliana* and *Solanum lycopersicum*. This alignment was performed using MUSCLE program (Edgar, 2004) [6] within the MEGA X software package. Subsequently, a phylogenetic tree was constructed using the neighbor-joining (NJ) method. To assess the robustness of the tree, a bootstrap test with 1000 replicates was employed. This entire process of alignment and phylogenetic tree construction was carried out using the MEGA X software (Kumar *et al*., 2018) [18].

Physiochemical Properties and Analysis of Conserved Motifs

The physiochemical parameters of PhFBP20 protein is calculated by ExPASy-ProtParam tool (http://web.expasy.org/protparam/), these parameters include amino acid, molecular weight, isoelectric point (pI), extinction coefficient, atomic composition, estimated half-life, grand average of hydropathicity (GRAVY), instability index and aliphatic index (Gasteiger *et al.*, 2005) [9]. The conserved motifs localized within the PhFBP20 and other related MADS protein sequences MEME (multiple expectation for motif elicitation) tool (http://meme-suite.org/tools/meme) was used with default parameters with 10 maximum motifs (Bailey *et al.*, 2015) [3].

Secondary structure and 3 D structure of protein

The PhFBP20 protein secondary structure and molecular modelling was predicted by PHYRE2 (Protein Homology structure prediction servers) (Kelley *et al*., 2015) [16] and available at http://www.sbg.bio.ic.ac.uk/phyre2. Based on the ranking of raw alignment score, the quantity and quality of aligned residues, offered the greatest query coverage and confidence. The 3-D model of PhFBP20 is shown by using RasMol software (Goodsell, 2005) [10].

Semi quantitative expression Analysis

Total RNA was successfully extracted from various tissues, including leaf, sepal, and petal, as well as from different stages of *Petunia hybrida* flowers, including initial bud (S1), closed flower (S2), and open flower (S3). This RNA served as the template for the synthesis of first-strand cDNA using reverse transcriptase (GeNeiTM in Bangalore). For semiquantitative expression analysis of PhFBP20 in *Petunia*

hybrida, PhFBP20 was detected with the Forward primer 5′-ATGGTGAGAGGAAAAACTC -3′ and Reverse primer 5′-CACCAATTAATTCTGTAAGCG -3′, while the β-Actin gene was used for cDNA normalization and amplified as a control with the Forward primer 5′-ACCACAGGTATTGTGTTGGACTC 3′ and Reverse primer 5′ AGAGCATATCCTTCATAGATGGG -3′. The PCR amplification was carried out by pre denaturation for 3 min at 94 °C, total 35 cycles, denaturation for 1min at 94 °C, annealing for 30s at 52 °C, synthesis for 1min at 72 °C and final extension for 10 min at 72 °C. The concern gene was examined at different cycles i.e., 21rd cycle, 24th cycle, 27th cycle, 30th cycle and 35th cycle for the expression analysis. PCR product was electrophoresed on 2% agarose gel. B-Actin housekeeping gene was selected as control to normalize the expression data, ensuring reliable and accurate analysis of PhFBP20 expression in *Petunia hybrida.*

Results and Discussion

Cloning and Sequence Analysis of PhFBP20 Gene

Total RNA was extracted from leaf of *Petunia hybrida* and quality of RNA was checked prior to cDNA synthesis using agarose gel electrophoresis. The PhFBP20 MADS Box gene was amplified from the cDNA of leaf samples. A coding region of around 651bp was amplified by PCR using PhFBP20 gene specific primers. The amplicon was visualized in 2% Agarose gel electrophoresis (Fig. 2). The amplified product was ligated into pGEM-T Easy cloning vector of size 3015 bp. Further confirmation of clone with insert was carried out by colony PCR and restriction digestion of the plasmid DNA using restriction enzyme EcoRI of the pGEM-T Easy vector whereas separation of the restricted products by 2% agarose gel electrophoresis produced 651bp band specific to PhFBP20 gene and ~3.0 kb band of restricted plasmid vector. The recombinant clone of PhFBP20 MADS Box gene was sequenced.

Fig 2: PCR amplification of PhFBP20 *Petunia hybrida* samples from leaf

Comparative analysis of *Petunia hybrida* and *Petunia axillaris* FBP20 gene

Multiple sequence alignment of the isolated PhFBP20 nucleotide sequence from *P. hybrida* with the FBP20 nucleotide of *Petunia axillaris* and available FBP20 nucleotide of *Petunia hybida* retrieved from NCBI revealed seven nucleotide change in the MADS Box domain in isolated PhFBP20 of *Petunia hybrida* and *Petunia axillaris*. No nucleotide change in isolated PhFBP20 and *Petunia hybrida* retrieved from NCBI (Fig. 2A). Multiple sequence alignment of the isolated PhFBP20 protein (translated by with the FBP20 protein of *Petunia axillaris* and *Petunia hybida* retrieved from NCBI revealed four amino acid change in the MADS Box domain. *Petunia hybrida* PhFBP20 and *Petunia axillaris* FBP20 i.e. the change in four amino acid residues Arginine (R) in place of Lysine (K; 9th), Arginine (R) in place of Glycine (G; 10th), Isoleucine (I) in place of Valine (V; 12th) and Glutamic acid (E) in place of (X; 13th) and there was no changes in amino acids of newly isolated and available FBP20 gene of *Petunia hybrida* (Fig. 2B).

Phylogenetic analysis

A phylogenetic analysis of the PhFBP20 gene was conducted, comparing it with MIKC-type MADS genes from *Arabidopsis thaliana* and *Solanum lycopersicum* (tomato). The analysis employed the neighbor-joining (NJ) method, and bootstrap values were generated from 1000 replicates to assess the reliability of the phylogenetic trees. The phylogenetic tree was constructed using nucleotide sequences of MIKC group MADS Box genes to elucidate the evolutionary relationship between *Petunia hybrida* PhFBP20 and other MIKC MADS box proteins. The results of the analysis indicated that PhFBP20 exhibits similarity to AGL20 in *Arabidopsis thaliana* and is closely related to SOC1-like and Agamous-like proteins found in *Solanum lycopersicum* (tomato). In another closely related clade, PhFBP20 shares similarity with transcription factors AGL14 and AGL19 in *Arabidopsis thaliana* and SOC-like (FYLF) proteins in *Solanum lycopersicum* shown in Fig 3. These findings provide insights into the evolutionary relationships and potential functional similarities of PhFBP20 with these related MADS box proteins.

Secondary structure and 3 D structure of protein

The secondary structure analysis of the PhFBP20 protein was performed using the PHYRE2 tool. This analysis predicted the distribution of secondary structure components within the protein. The results indicated that PhFBP20 consists of approximately 45% alpha helix, 6% beta sheets, and 35% disordered regions, as illustrated in Figure 4. PhFBP20 protein modeling was carried out using the PHYRE2 tool, which enables the prediction and analysis of protein structure and function. In this process, the target sequence was subjected to PSI-BLAST within PHYRE2, which helps identify evolutionary relationships between homologous sequences. The PhFBP20 protein sequence was submitted to PHYRE2, resulting in the generation of a 3-D protein model with a high confidence level of 99.9%. The template that best suited the generation of the protein model was c7nb0A, representing a developmental protein called sepallata 3. The modeled structure of PhFBP20 was visualized using the RasMol tool, as depicted in Figure 6A. In a previous study, MADS-box genes HsMADS1 and HsMADS2 were isolated from Hibiscus sabdariffa, and their 3-D models were visualized using RasMol. This study also identified alpha helix-rich structures within the deduced amino acid sequences of HsMADS1 and HsMADS2. Furthermore, quality assessment and alignment confidence checks were conducted on the PhFBP20 protein model using PHYRE2, as demonstrated in Figure 5A and Figure 5B. These analyses ensure the reliability and accuracy of the generated protein model.

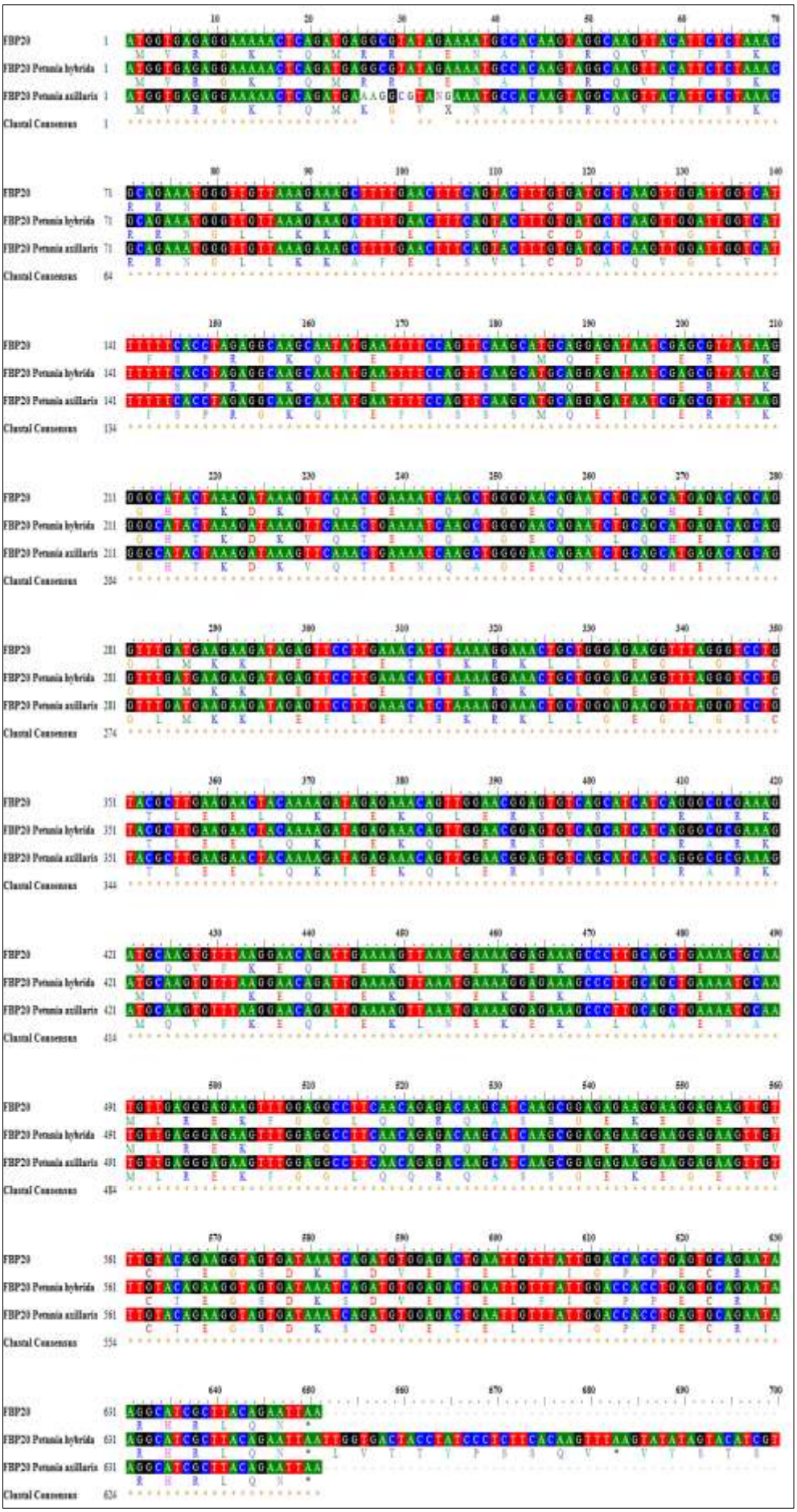

Fig 2A: Comparative analysis of nucleotide sequence of isolated PhFBP20 from *Petunia hybrida* with *Petunia hybrida* and *Petunia axillaris* FBP20 retrieved from NCBI database

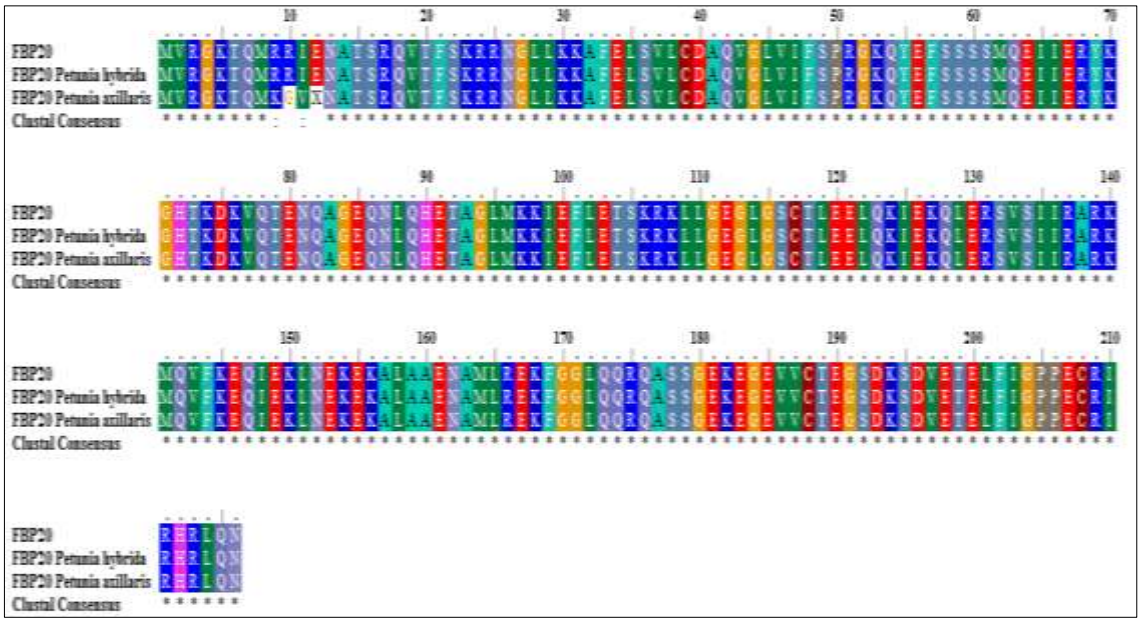

Fig 2B: Comparative analysis of amino acid sequence of isolated PhFBP20 from *Petunia hybrida* with *Petunia hybrida* and *Petunia axillaris* FBP20 retrieved from NCBI database

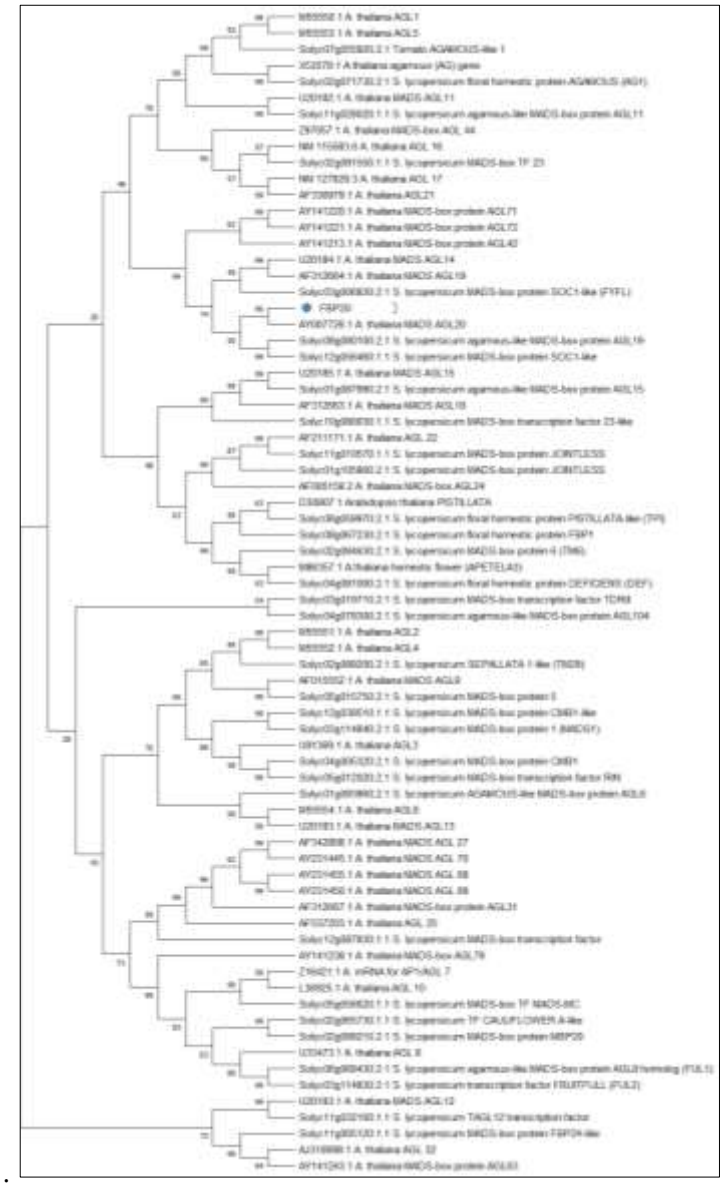

Fig 3: Phylogenetic tree of PhFBP20 with *MIKC MADS* box TF from *Arabidopsis thaliana* and *Solanum lycopersicum*

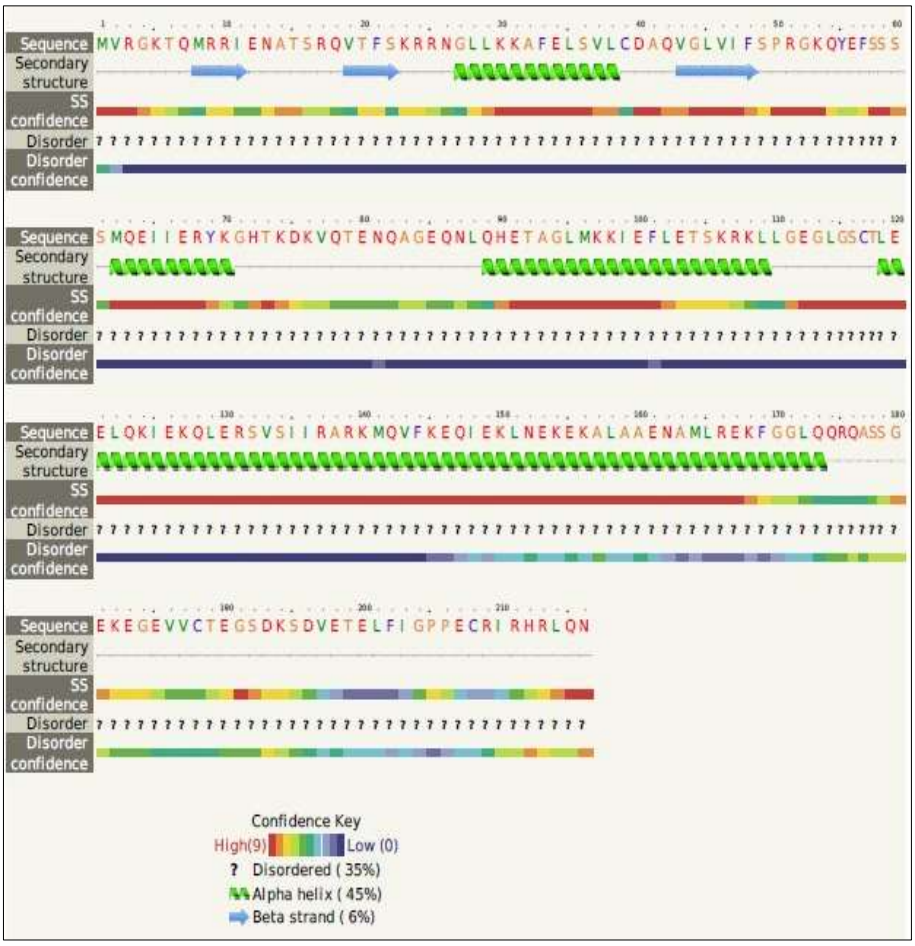

Fig 4: Secondary structure analysis of PhFBP20 using Phyre2

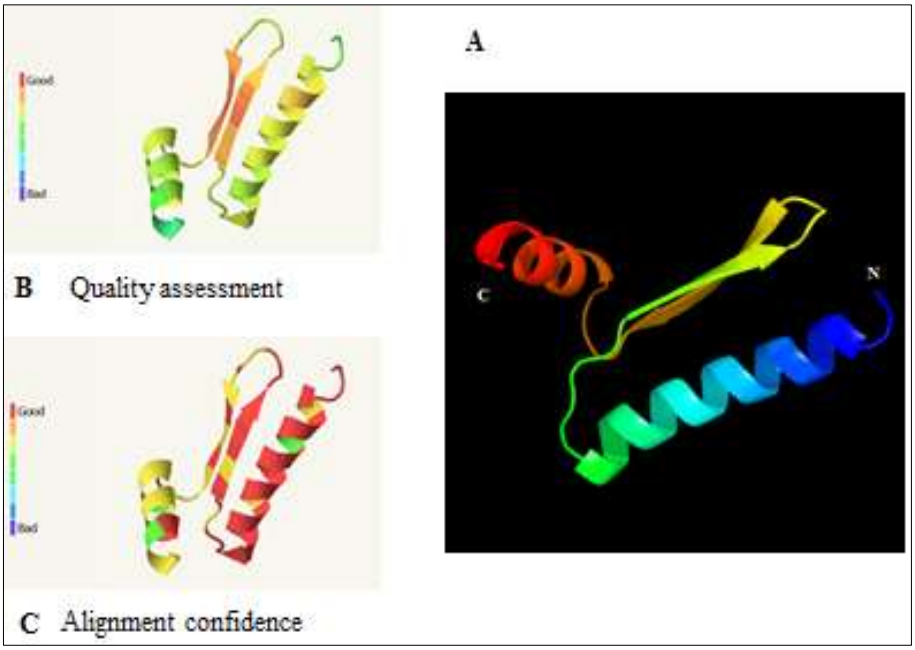

Fig 5: Structure modelling of the PhFBP20 protein A. Structure prediction of PhFBP20 protein constructed by using Phyre2. N-terminal, alpha helix and C-terminal are labelled B. Quality assessment C. Alignment confidence

Physiochemical Properties and Analysis of Conserved Motifs

The ExPASy-ProtParam tool was employed to analyze the primary structure of the PhFBP20 protein and compute various physical and chemical parameters, as summarized in Table 2. The molecular weight of PhFBP20 was determined to be approximately 24,649.22 KD (kiloDaltons). The isoelectric point (pI) was found to be 9.23, indicating a basic nature for the protein, as this is the pH at which the protein carries no net charge. The aliphatic index, which reflects the relative abundance of aliphatic residues compared to aromatic residues, was calculated to be 75.83. This high aliphatic index

suggests that the protein is rich in aliphatic residues, contributing to its stability over a wide range of temperatures. The instability index, with a value of 56.56 for PhFBP20, indicates that the protein is considered unstable since it exceeds the threshold of 40. The GRAVY (Grand Average of Hydropathicity) value of -0.769 suggests that the protein is hydrophilic in nature, indicating good water solubility. Hydrophilic proteins interact well with water, and a negative GRAVY score signifies hydrophilicity. The nucleotide composition of PhFBP20 was determined using BioEdit software, revealing the presence of adenine in the highest abundance and cytosine in the lowest abundance. The translation of the PhFBP20 nucleotide sequence resulted in a protein sequence consisting of 216 amino acid residues. The amino acid composition analysis indicated that glutamic acid had the highest molecular percentage, while tryptophan had the lowest molecular percentage. These parameters collectively provide insights into the physicochemical characteristics and properties of the PhFBP20 protein.

The search for conserved motifs within the MADS Box PhFBP20 TFs protein sequences of *Petunia hybrida* and closely related MIKC type MADS box proteins of *Arabidopsis thaliana* and *Solanum lycopersicum* was conducted using the Multiple Expectation for Motif Elicitation (MEME) tool version 5.3.0. A total of 10 motifs were identified in the MADS box sequences, with all query sequences sharing the Signature Domain of the MADS Box family, which includes the MADS box and K-box domain (Motif 1 and 2).

Motifs 3, 4, 5, and 6 were also present in all query sequences, suggesting their conservation across the dataset. Motif 7 was observed in all query sequences except for AGL14 of *Arabidopsis thaliana* and SOC1-like of *Solanum lycopersicum* Solyc03g006830.2.1. Motifs 8 and 9 were found in AGL14 and AGL19, while motif 10 was exclusively present in AGL19 of *Solanum lycopersicum* Solyc08g080100.2.1 (as shown in Fig 6). These findings indicate that a total of 10 conserved motifs were identified within the dataset of 51 MIKC protein sequences. Within the DlMADS subfamily, motifs were distributed in a range from 1 to 7, and each subfamily exhibited similar motif compositions. Notably, motifs 1 and 4 were identified in most protein sequences, highlighting their potential functional significance. Motifs 2, 3, and 7 appeared to be fragments of the K-box and were prevalent among most MIKCC members, while motif 5, typically associated with the K-box, was exclusively found within the MIKC* class. he result reveals that the primary differences between MIKCC and MIKC* proteins are variations in the K domain (Wang *et al*., 2022) [30]

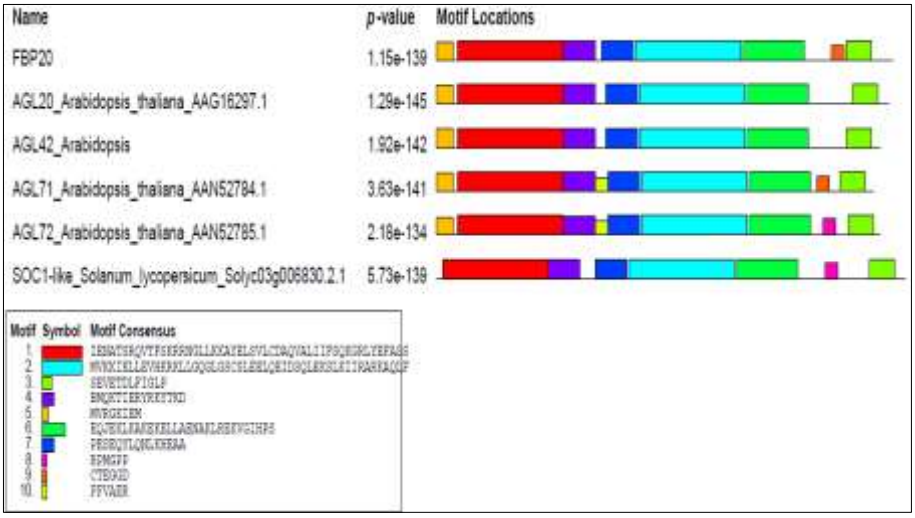

Fig 6: Motif analysis of PhFBP20 MADS-box gene in Petunia and other MADS-Box genes from different plant species, Motif compositions: Protein sequences are indicated by thick grey lines, different colored boxes represent the conserved motifs

Semi Quantitative expression analysis of PhFBP20

A semi-quantitative expression analysis of PhFBP20 was conducted in three different tissues (leaf, sepal, and petal) and three distinct flower stages (Initial bud: S1, mature bud: S2, and open flower: S3) of *Petunia hybrida*, revealing varying expression patterns. The expression of PhFBP20 was notably higher in S1 (Initial bud) and leaf tissues but lower in the petal tissue. As illustrated in Figure 7.1, the gene expression exhibited a down-regulation trend from S1 (initial bud) to S3 (Open flower), with an approximately 8-fold decrease in expression levels in both S3 (initial bud) and S3 (open flower) compared to S1 (Initial bud). Similarly, the gene expression demonstrated a down-regulation pattern from leaf to sepal tissues, with an approximately 8-fold decrease in expression levels in sepal tissue and very low expression levels observed in petal tissue compared to leaf tissue, as shown in Figure 7.2. These findings align with previous studies conducted by Sun *et al*. (2014) [31] and Tang *et al*. (2016) [32], which reported higher expression levels in young flower buds compared to mature flower buds for AP1 orthologs, such as CjAPL2 (C. japonica) and JcAP1 (J. curcas). Additionally, Immink *et al*. (2003) [33] observed strong expression of FBP20, FBP21, and FBP28 genes in the leaves of *Petunia hybrida*. Ferrario *et al*. (2004) [34] also reported that FBP20 expression was prominent in vegetative apices but decreased following the transition to flowering, further supporting the observed expression patterns of FBP20 in different tissues and flower stages.

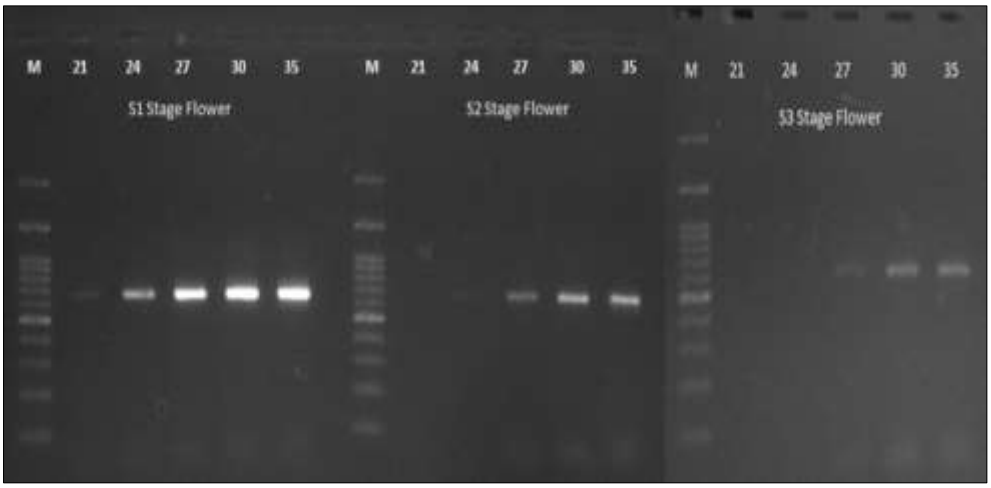

Fig 7: Semi-Quantitative Expression profile of PhFBP20 of *Petunia hybrida* at three different stages of flower *viz*., initial bud: S1, maturebud: S2 and open flower: S3. M-Marker, 100bp: (Genei), Amplification at PCR cycle 21-35.

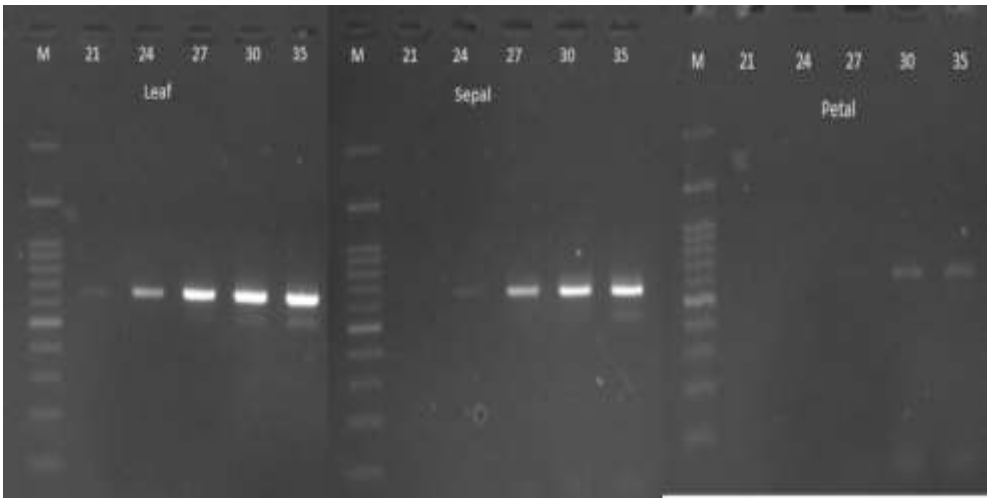

Fig 8: Semi-Quantitative Expression profile of PhFBP20 of *Petunia hybrida* at three different flower tissue *viz*., leaf, sepal and petal. M-Marker, 100bp: (Genei), Amplification at PCR cycle 21-35.

Conclusion

In our study, we reported a variable expression of the PhFBP20 gene at different stages of flower development and found the higher expression in the initial bud compared to the mature bud and open flower. Conversely, the expression was higher in the leaf than in the sepal, and lower expression was observed in the petals. This pattern of gene expression suggests that PhFBP20 plays a role in transition of vegetative bud meristem to floral bud and flower development. Sequence analysis of the isolated PhFBP20 gene from our *Petunia hybrida* and the PhFBP20 gene from *Petunia axillaris*, retrieved from NCBI, revealed four amino acid sequence differences between them. However, based on the secondary structure analysis and prediction of the MADS Box domain of the PhFBP20 gene, we determined that these amino acid changes occurred in both β strands and in disordered regions. Importantly, these variations in the amino acid sequence did not result in any changes in the 3-D structure or the function of the MADS Box domain of the protein under investigation. Furthermore, our phylogenetic analysis indicated that PhFBP20 is closely related to the AGL20 transcription factor of *Arabidopsis thaliana*. These findings collectively suggest that PhFBP20 is involved in flower development and shares structural and functional similarities with related transcription factors in other plant species, such as *Arabidopsis thaliana*.

Acknowledgement

We are thankful to the Department of Agricultural Biotechnology, SVPUA&T, Meerut for providing necessary facilities.

References

1. Alvarez-Buylla ER, Pelaz S, Liljegren SJ, Gold SE, Burgeff C, Ditta GS, *et al*. An ancestral MADS-box gene duplication occurred before the divergence of plants and animals. Proceedings of the National Academy of Sciences. 2000;97(10):5328-5333.
2. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, *et al*. MEME SUITE: tools for motif discovery and searching. Nucleic acids research. 2009;37(2):W202-W208.
3. Bailey TL, Johnson J, Grant CE, Noble WS. The MEME suite. Nucleic acids research. 2015;43(W1):W39-W49.
4. Becker A, Theißen G. The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Molecular phylogenetics and evolution. 2003;29(3):464-489.
5. Cao Y, Wang L, Zhao J, Zhang H, Tian Y, Liang H, *et al*. Serum response factor protects retinal ganglion cells against high-glucose damage. Journal of Molecular Neuroscience. 2016;59:232-240.

6. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids research. 2004;32(5):1792-1797.
7. Fan CM, Wang X, Wang YW, Hu RB, Zhang XM, Chen JX, *et al*. Genome-wide expression analysis of soybean MADS genes showing potential function in the seed development. PloS one. 2013;8(4):e62288.
8. Garay-Arroyo A, Ortiz-Moreno E, de la Paz Sánchez M, Murphy AS, García-Ponce B, Marsch-Martínez N, *et al*. The MADS transcription factor XAL2/AGL14 modulates auxin transport during Arabidopsis root development by regulating PIN expression. The EMBO journal. 2013;32(21):2884-2895.
9. Gasteiger E, Hoogland C, Gattiker A, Duvaud SE, Wilkins MR, Appel RD, *et al*. Protein identification and analysis tools on the ExPASy server Humana press; c2005. p. 571-607.
10. Goodsell DS. Representing structural information with RasMol. Current Protocols in Bioinformatics. 2005;11(1):5-4.
11. Gramzow L, Ritz MS, Theißen G. On the origin of MADS-domain transcription factors. Trends in Genetics. 2010;26(4):149-153.
12. Hall T, Biosciences I, Carlsbad CJGBB. BioEdit: an important software for molecular biology. GERF Bull Biosci. 2011;2(1):60-61.
13. Inukai S, Kock KH, Bulyk ML. Transcription factor–DNA binding: beyond binding site motifs. Current opinion in genetics & development. 2017;43:110-119.
14. Jarvis EE, Clark KL, Sprague GF. The yeast transcription activator PRTF, a homolog of the mammalian serum response factor, is encoded by the MCM1 gene. Genes & development. 1989;3(7):936-945.
15. Kaufmann K, Melzer R, Theißen G. MIKC-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. Gene. 2005;347(2):183-198.
16. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nature protocols. 2015;10(6):845-858.
17. Knapp S, Bohs L, Nee M, Spooner DM. Solanaceae-a model for linking genomics with biodiversity. Comparative and functional genomics. 2004;5(3):285-291.
18. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution. 2018;35(6):1547.
19. Norman C, Runswick M, Pollock R, Treisman R. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fos serum response element. Cell. 1988;55(6):989-1003.
20. Parenicova L, De Folter S, Kieffer M, Horner DS, Favalli C, Busscher J, *et al*. Molecular and phylogenetic analyses of the complete MADS-box transcription factor family in Arabidopsis: New openings to the MADS world. The Plant Cell. 2003;15(7):1538-1551.
21. Passmore S, Maine GT, Elble R, Christ C, Tye BK. Saccharomyces cerevisiae protein involved in plasmid maintenance is necessary for mating of MATα cells. Journal of molecular biology. 1988;204(3):593-606.
22. Riaño-Pachón DM, Ruzicic S, Dreyer I, Mueller-Roeber B. PlnTFDB: an integrative plant transcription factor database. BMC bioinformatics. 2007;8(1):1-10.
23. Shilpy Singh, Vivek Rana, Pushpendra Kumar, Manoj Kumar Yadav, Vaishali. Isolation and Expression Analysis of Floral Binding Protein 20 (FBP20) MADS Box Gene in *Petunia axillaris*. Int. J Curr. Microbiol. App. Sci. 2019;8(01):1942-1955.
24. Smaczniak C, Immink RG, Angenent GC, Kaufmann K. Developmental and evolutionary diversity of plant MADS-domain factors: Insights from recent studies. Development. 2012;139(17):3081-3098.
25. Sommer H, Beltran JP, Huijser P, Pape H, Lönnig WE, Saedler H, *et al*. Deficiens, a homeotic gene involved in the control of flower morphogenesis in Antirrhinum majus: the protein shows homology to transcription factors. The EMBO Journal. 1990;9(3):605-613.
26. Thangavel G, Nayar S. A survey of MIKC type MADS-box genes in non-seed plants: algae, bryophytes, lycophytes and ferns. Frontiers in plant science. 2018;9:510.
27. Theissen G, Kim JT, Saedler H. Classification and phylogeny of the MADS-box gene families in the morphological evolution of eukaryotes. J Mol Evol. 1996;43(5):484-516.
28. Vandenbussche M, Chambrier P, Rodrigues Bento S, Morel P. Petunia, your next supermodel?. Frontiers in plant science. 2016;7:72.
29. Yanofsky MF, Ma H, Bowman JL, Drews GN, Feldmann KA, Meyerowitz EM. The protein encoded by the Arabidopsis homeotic gene agamous resembles transcription factors. Nature. 1990;346(6279):35-39.
30. Wang W, Xie E, Li X, Fan DP, Song K, Liang D, Lu T, Luo P, Shao L. Pvt v2: Improved baselines with pyramid vision transformer. Computational Visual Media. 2022 Sep;8(3):415-24.
31. Sun M, Qu S, Hao Z, Ji W, Jing P, Zhang H, *et al*. Towards efficient solid-state photoluminescence based on carbon-nanodots and starch composites. Nanoscale. 2014;6(21):13076-81.
32. Tang H, Hammack C, Ogden SC, Wen Z, Qian X, Li Y, *et al*. Zika virus infects human cortical neural progenitors and attenuates their growth. Cell stem cell. 2016 May 5;18(5):587-90.
33. Immink RG, Ferrario S, Busscher-Lange J, Kooiker M, Busscher M, Angenent GC. Analysis of the petunia MADS-box transcription factor family. Molecular Genetics and Genomics. 2003 Feb;268:598-606.
34. Ferrario S, Busscher J, Franken J, Gerats T, Vandenbussche M, Angenent GC, *et al*. Ectopic expression of the petunia MADS box gene UNSHAVEN accelerates flowering and confers leaf-like characteristics to floral organs in a dominant-negative manner. The Plant Cell. 2004 Jun 3;16(6):1490-505.