

GENOME WIDE ANALYSIS OF FINGER MILLET JMJ-C GENE FAMILY AND EXPRESSION PROFILE STUDIES IN RESPONSE TO ABIOTIC STRESS

PROJECT WORK

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

By

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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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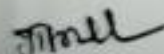
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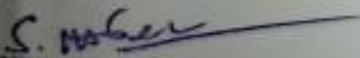
BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **AISHWARYA.S (40230001)** and **SNEHA.G.K (40230043)** who carried out Project entitled "**Genome Wide Analysis of Finger Millet JMJ-C Gene Family and Expression Profile Studies in Response to Abiotic Stress**" under my supervision from October 2023 to March 2024.


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DECLARATION

We, **AISHWARYA.S (40230001)** and **SNEHA.G.K(40230043)**, hereby declare that the Project Report entitled “**GENOME WIDE ANALYSIS OF FINGER MILLET JMJ-C GENE FAMILY AND EXPRESSION PROFILE STUDIES IN RESPONSE TO ABIOTIC STRESS**” done by us under the guidance of Dr.J.Theboral at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

The finger millet (*Eleusine coracana*), a grain crop valued for its high nutritional content, is facing more difficulties as a result of shifting weather patterns. In order to improve the plant's resilience and guarantee food security, it is essential to comprehend its adaptive responses to abiotic stress. We used a multidisciplinary strategy in this extensive study to look into the molecular and physiological components of finger millet's response to salinity, heat, and drought stresses. Regulated stress conditions were applied to thirty-day-old finger millet seedlings for four days which simulating situations that are pertinent to current agricultural issues. To understand the plant's physiological responses to stress, a number of assays were carried out on carefully gathered tissue samples. Proline assays served as markers of stress-induced metabolic alterations, weight measurements revealed growth dynamics, and pigment analysis shed light on photosynthetic activities. A comparable study was conducted at the molecular level to determine the genetic underpinnings of stress reactions. Jumonji C (JMJC) genes were evaluated for their expression patterns using RT-PCR. These genes are known to be involved in molecular pathways associated to stress. The objectives of this genome analysis were to pinpoint important genes linked to stress tolerance and to clarify the regulatory networks that plan finger millet's adaptive responses. The complicated links between gene expression and physiological changes in response to abiotic stress were revealed by the combination of molecular and physiological data. Interestingly, dynamic expression patterns were seen for particular JMJC genes, indicating their critical function in stress adaptation mediation. This comprehensive method offers a sophisticated comprehension of the relationship between the molecular mechanisms and the physiological adaptations made by the plant during stress conditions. The discovery of potential stress tolerance-related genes creates opportunities for focused breeding initiatives to create stress-tolerant finger millet cultivars. In addition to ensuring consistent harvests, these cultivars would support agriculture's sustainability in the face of climatic uncertainty. In summary, this study adds to our understanding of the adaptation mechanisms of finger millet under abiotic stress and provides insightful information for next agricultural development initiatives. The integrated strategy, which combines molecular and physiological investigations, is an excellent example of a sound methodology for deciphering the intricate stress responses in significant cereal crops such as finger millet.

Keywords: Finger millet, Abiotic stress, Molecular responses, crop resilience

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LIST OF ABBREVIATIONS

ABRE: Absciscic acid response element

AA: Amino acid

ARE: Anaerobic induction

CRE: cAMP Response Element

Chl: Chlorophyll

CDS LENGTH/BP: Coding Sequence Length in Base Pairs

D1Dr1: Day 1 drought treated plants

D1H1: Day 1 heat treated plants

D1S1: Day 1 salt treated plants

D2Dr2: Day 2 drought treated plants

D2H2: Day 2 heat treated plants

D2S2: Day 2 salt treated plants

D3Dr3: Day 3 drought treated plants

D2H3: Day 3 heat treated plants (Corrected from previous response)

D3S3: Day 3 salt treated plants

D4Dr4: Day 4 drought treated plants

D4H4: Day 4 heat treated plants

D4S4: Day 4 salt treated plants

DNA: Deoxyribonucleic acid

DW: Dry weight

E.coracana: Eleusine coracana

FW: Fresh weight

pI: Isoelectric point

Jmj-C: Jumonji C

LRE: Light-Responsive element

LTR: Low-temperature responsive

mRNA: Messenger RNA

MeJA: Methyl jasmonate

Mw: Molecular weight

PEG: Polyethylene glycol

ROS: Reactive oxygen species

RWC: Relative water conte

RT-PCR: Reverse transcriptase polymerase chain reaction

RT-qPCR: Reverse Transcription Quantitative Polymerase Chain Reaction

RNA: Ribonucleic Acid

NaCl: Sodium chloride

TFs: Transcription factors

TW: Turgid weight

W-BOX: Wounding and pathogen responsive

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CHAPTER 1

INTRODUCTION

The term "genome-wide analysis" describes the investigation and examination of an organism's whole genome. An organism's whole genetic makeup, comprising all of its genes and non-coding sections, is called its genome. Rather than concentrating on certain genes or areas of the genome, genome-wide analysis looks at and interprets different parts of the entire genome.

JMJ-C gene is elaborated as jumonjiC and it was first discovered in mice using the gene trap technique. It was given the name "jumonji" in honor of the cruciform-looking abnormality that was seen in the neural plates of MJM-C mutant mice. These proteins catalyze various oxidative processes and they have the conserved MJM-C domain in all living organisms. The MJM-C gene family, which is distinguished by the conserved Jumonji C (MJM-C) domain, plays a crucial role in the control of epigenetics and chromatin remodelling in plants due to their wide range of biological effects. This family of proteins is broken up into seven distinct subgroups based on domain architecture and their ability to antagonize specific histone methylation marks. Their biological functions derive from their ability to regulate gene expression and include roles in cell differentiation, growth, proliferation, and stress responses (*Li et al., 2020*)

The control of gene activity that occurs without affecting the underlying DNA sequence is known as epigenetic regulation. Rather, it entails altering DNA's structure or the proteins that it interacts with. These changes can modify how genes are activated or inactive, which can have an impact on a range of cellular functions and processes. Histones are a class of proteins that play a crucial role in the structure and organization of DNA in the cell nucleus. They act as spools around which DNA is wound, helping to package the long DNA molecules into a more compact and organized structure called chromatin. Chromatin further condenses to form chromosomes during cell division (*Dutta et al., 2017*). A class of extremely conserved proteins known as core histones is essential to the arrangement and structure of DNA in eukaryotic cells. Core histones include H2A, H2B, H3, and H4

aid in compact structure and organization of genetic material. These histones are the building blocks of nucleosomes, which are the fundamental repeating units of chromatin. Together, they form the octameric protein complex known as the histone octamer.

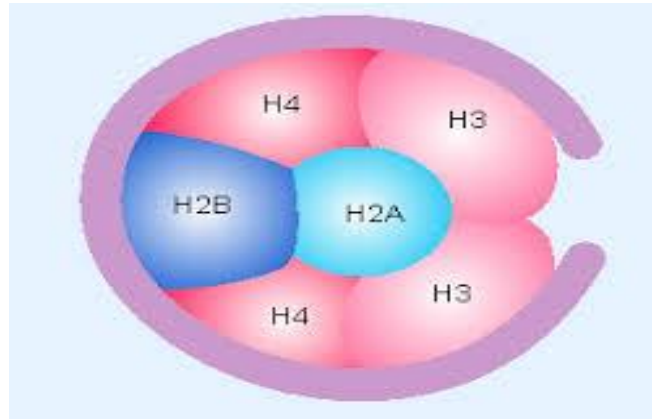


Fig1.1 Structure of histone octamer in a symmetrical model

In addition, their post translational modifications such as phosphorylation, methylation, acetylation, sumoylation, ubiquitination and ADP-ribosylation enable them to play major roles in epigenetic regulation. This regulatory system employs reversible chemical alterations to DNA and histones within eukaryotic chromatin to dynamically regulate overall gene expression. The amino acid residues of histone proteins particularly lysine, arginine, threonine and serine undergo any one of these modifications and contribute in gene regulations which together make up the "histone code".

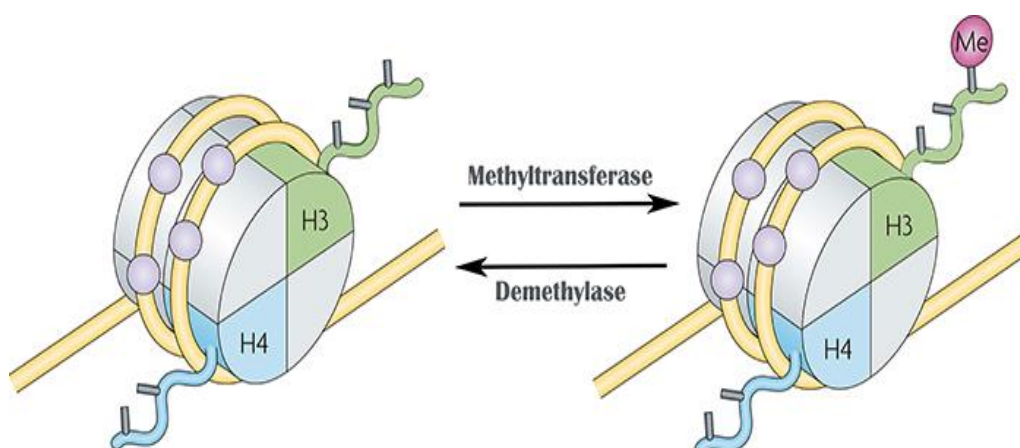


Fig 1.2 Methylation and demethylation of histone proteins

Of this, histone methylation has a significant impact on gene transcription and chromatin structure and heredity. Histone lysine methyltransferases (KMTs) from three protein families mediate histone lysine methylation, a significant participant of this process. Histone changes orchestrate the way that plants react to environmental challenges like cold, salinity, drought, and submersion. In plants, DNA methylation is tightly correlated with histone H3K9 methylation, an epigenetic marker of gene inactivation. There are two categories of demethylase are involved in the homeostasis of methylation in organisms. They are monoamine oxidase (MAO) homolog, Lysine Specific Demethylase 1 (LSD1/KDM1) JMJ-C domain containing histone demethylases. Among these categories, JMJ-C domain containing histone demethylases are explored recently and gains much attention due to their versatile roles in regulatory mechanisms. It is noted that the JMJ-C protein contains the conserved domains of JMJ-C as well as JmjN. The AT-Rich interaction domain (ARID) and the JmjN domain were further separated from one another. The JMJ-C domain was discovered to be another conserved domain in addition to ARID which is a DNA binding domain. These proteins are a class of histone lysine demethylases (KDMs). These enzymes demethylate lysine residues in macromolecules like proteins and nucleic acid.

Unlike some other histone demethylases, JMJ-C domain containing proteins can reverse all three states of lysine methylation: mono-, di- and tri- methylation. Since plant JMJ-C proteins are explored with limited plant species, we tried to highlight their importance through analyzing their functions in gene regulation (*He et al., 2021*). *Eleusine coracana*, or finger millet, is a vital cereal crop within the *Poaceae* family, prized for its highly nutritious grains. Belonging to the kingdom *Plantae*, it falls under the phylum *Angiosperms* and class *Monocots*. Its scientific classification includes the order *Poales*, family *Poaceae*, genus *Eleusine*, and species *E.coracana*. It is a highly nutritious crop that is widely grown in South Asia and Africa. It is abundant in fibre, protein, and other necessary amino acids as well as vitamins and minerals. Notably free of gluten, it can be utilised in a variety of culinary applications, such as porridge and flatbreads (*Xu et al., 2015*).

Finger millet is well renowned for its ability to withstand abiotic challenges including drought and unfavourable soil. Research into the JMJ-C gene in ragi may shed light on the genetic processes that support the plant's ability to withstand stress. In some

areas, ragi is a staple crop, and enhancing its resilience and guaranteeing food security require an understanding of how it reacts to abiotic stress at the genetic level. Finger millet has high nutritional value and is a valued crop. Examining the function of the JMJ-C gene in stress response may help improve the crop's agronomic qualities, such as nutritional value, in challenging environments. (*Meng et al., 2018*) Researchers investigate how plants respond to environmental stressors through the use of abiotic stress and RT-PCR. Abiotic stressors are applied to plants to simulate unfavourable conditions, such as drought, salt, or extremely high or low temperatures. RT-PCR is then used to examine variations in the transcription levels of particular genes (*Shi et al., 2023*).

CHAPTER 2

.LITERATURE SURVEY

2.1 ASPECTS OF JMJ-C GENE FAMILY:

As discussed previously, the dynamics, structure and function of genome are greatly impacted by modification to the histone molecules in chromatin. For instance, methylation of both lysine and arginine residues contributes in a variety of functions, including heterochromatin transcription control, X- chromosome inactivation, and formation (*Dong et al., 2020*). Moreover, it was evident that for the control of transcription, the preservation of epigenetic information, and the determination of cell fate, Histone lysine methylation is particularly crucial (*Fan et al., 2012*).

In contrast to acetylation, histone lysine methylation can indicate either activation (or) repression occurs during regulation. In this aspect, JMJ-C domain containing proteins regulate growth and development of plant at many stages including when it blooms, develops male and female components (*Han et al., 2016*). It was also discovered that the genes contain responsive elements to signals from hormones, light and stress (*He et al., 2021*). Most importantly, they are the part of the plant biological clock and has a role in controlling the circadian rhythm, particularly the length of the circadian rhythm (*Hong et al., 2007*). Of the JMJ-C -domain only proteins, FIH domain is one of the more extensively researched. It has been demonstrated to be crucial in reactions to hypoxia process (*Khan et al., 2023*).

2.2 JMJ-C GENE FAMILY MEMBERS:

The family has been classified into seven subfamilies based on their sequences. These includes JHDM1, PHF2/PHF8, JHDM2, UTX/UTY, JHJDM3, JARID and JMJ-C domain only. The first JMJ-C continuously protein shown to demethylate histone protein peptide is JHDM1 and particularly introducing that JHDM1 and JMJD2, despite JMJD2 as weakness could perform a identical reaction, the demethylation of H3-k36me₂ to H3-k36me. PHF2 suppresses the activity of the rDNA gene by binding to di-/tre methylated histone 3lysine 4(H3K KMe_{2/3}) competing with PHF8 for rDNA promoter binding, recruiting SUV3941 to add repressive H3K9me_{2/3} makes the represented specific genes like RAR, oct 4, PHF8 stimulated the transcription of particular genes and aids in the regulation of gene expression by activating rDNA transcription, promoting

H3K4Me2/3 binding and interacting with PHF2 to affect promoter binding and activating transcription for specific genes. Histone H3K9Me2 and H3K9Me1 methyl marks can be removed by JHDM2 proteins. The JMJC domain containing protein known as UTX (ubiquitously transcribed tetratricopeptide repeat X chromosome) functions as a histone demethylase, primarily removing methyl marks from H3K27. For epigenetic regulation, preventing polycomb mediated gene silence and possibly affecting X chromosome inactivation and dynamically modifying the gene expression by histone modification this demethylation activity is essential. The JHDM3 proteins could control transcription of these proteins known as H3K9Me2/3 and H3K36me2/3 are histone demethylase that may remove methyl groups from histone H3 at particular locations. JARID1 specifically targets the methylated lysine residual H2K4Me3, H3K4Me2 and H3K4Me1 in the histone H3. These protein functions to demethylate histones which helps in the dynamic regulations of gene expression. Based on the presence of particular amino acids required for their demethylation activity the majority of these proteins are most likely active histone demethylase. The precise substances they target and their roles in gene regulation, however are yet unknown and need more research (Li et al., 2023).

2.3 GENOME-WIDE ANALYSIS OF JMJC FAMILY GENES IN PLANTS:

Transcriptomics, also known as genomic transcription analysis, refers to the investigation of gene expression at the genome level. This field allows researchers to pinpoint which genes or transcript isoforms are more abundant in specific cell types, tissues, conditions, diseases, or phenotypes. This analysis is conducted using data derived from techniques such as microarrays or high-throughput sequencing (Li et al., 2022). The HMM-based method consistently outperforms the BLAST-based method in terms of identifying more members within gene families. So far, JMJC family genes were studied in wheat, maize, mung bean, moso bamboo, birch, grape, tomato, Arabidopsis, rice, sweet orange, soybean and alfalfa (Li et al., 2023).

A phylogenetic tree using a multiple sequence alignment was performed in the green plants both eudicotyledonous and monocotyledonous plants had the majority of JMJC genes in various subfamilies (Hong et al., 2007). A total of 24 JMJC genes were identified in wheat and distributed into two subfamilies (Mali et al., 2023). Nineteen JHJDM proteins in maize were discovered and were divided into three distinct subfamilies based on structural traits (Panara et al., 2022). A total

of 18 VrJmj genes were discovered in mungbean while 22 genes were found in moso bamboo and were grouped into 4 subfamilies (Qian et al., 2019), (Shi et al., 2014) and 21 JMJC genes were found in the birch, Arabidopsis (Shi et al., 2023), (Ma et al., 2022) 20 were found tomato, sweet orange and rice (Li et al., 2023), (Alves et al., 2022), (Ma et al., 2022) while 19 were found in peach (He et al., 2021). Twenty-seven genes were found in *Taraxacum kok-saghy* (Shi et al., 2014), 34 genes were found in *Medicago truncatula*, 26 genes were found in potatoes (Takeuchi et al., 2006) and 50 genes were found *Gossypium hirsutum* (Tsukada et al., 2005). All of them are categorized into 5 subfamilies. Fifty-one genes were predicted in the cotton while 20 genes were predicted in litchi and both were categorized into 6 subfamilies each (Tian et al., 2021), (Wang et al., 2022). Sixty-five genes were predicted in *Brassica napus* while 12 genes in *Rosa chinensis* and both were grouped into 7 subfamilies each (Xu et al., 2015), (Zhang et al., 2023).

2.4 CIS-ELEMENT ANALYSIS AND THEIR COMPARITIVE STUDIES:

Cis-acting elements are unique DNA sequences located in the promoter regions of genes. By interacting with transcription factors and other regulatory proteins, they play a critical role in controlling gene expressions (Hong et al., 2007). Studies emphasize that histone demethylases with JMJC domains play a critical part in the response to stress and light by altering transcription factors and phenotype-specific genes (Mali et al., 2023). In JMJC gene members, totally 13 kinds of Cis-regulatory elements (CREs) were identified and categorized into three main categories: stress-related, hormone-related and light response-related. The JMJC genes' promoter regions also contain cis-acting components linked to the control of metabolism and cellular development. These components probably regulate the expression of genes involved in basic cellular functions, growth, and metabolism (Hong et al., 2007). The total number of CREs are different for each plant and the majority of the elements were linked to light response. and In birch, the LTR factor was found to suppress the gene expression in response to low temperature outside (Shi et al., 2023). light and stress response elements were found in the promoter after cis-element analysis in potato and grapes (He et al., 2021), (Takeuchi et al., 2006). Twelve distinct elements connected to various environmental pressures were discovered when the promoter region in 11 different types of rice species was analyzed. The anaerobic environment, the plant hormone ABA, MYB transcription factors, heat shock and general stress response were some of the most prevalent components. They discovered that the rice species *O. nivara* had the gene promoters with the

highest average concentration of these stress-related components (Zhou & Ma, 2008). In addition to CREs, the putative regulatory transcription factors (TFs) and their binding sites in JMJs were also examined in some plants. Analysis revealed that several significant plant TFs, including AP2/ERF, MYB, C2H2, Nin-like transcription factors, and DNA binding with one finger (Dof) and NAC were found. Since, AP2/ERF and MYB, are widely acknowledged to play crucial regulatory functions in plant growth, differentiation, and metabolism, it is presumed that JMJ gene members may play a vital role in such processes (Tian *et al.*, 2021). Other than this, several transposable elements (TEs) including DNA transposons, Non-LTR retroelements (LINE elements) and LTR retro elements are also identified in JMJ family members (Zhang *et al.*, 2020). In Moso bamboo, multiple genes were affected by treatments with abscisic acid (ABA) and Methyl Jasmonate (MeJA), suggesting that these genes may play a part in hormone mediated reactions (Shi *et al.*, 2014). The analysis in *G.hirsutum* contained elements linked to hormones (ABRE, CRE, CGTCA motifs, AUXRR-core, GARE) and a variety of stressors (LTR, MBS Myb, G-box GT1-Motif, circadian, ARE) (Tsukada *et al.*, 2005). In Tormentosa, promoters of 18 CgJMJC genes were searched and 15 of them had ABRE (Zaidi *et al.*, 2018).

2.5 COMPARITIVE STUDIES OF EXPRESSION PATTERNS OF JMJC GENES:

The investigation with different stresses such as cold, heat, drought and hormones revoked versatile number of individual genes and family of JMJC genes. They exhibited differential expression with no relational factors. For instance, in birch, after an hour of cold stress, eleven genes were up-regulated while 10 genes, particularly all the members of JHDM3 /KDM4 were down-regulated among 21 members. The study concluded that there was a negative correlation with cold stress and LTR elements (Shi *et al.*, 2023). When analyzing the reports we found that, heat stress did not generate significant impact in case of birch, whereas in maize seven ZmJMJC genes, showed significant upregulation (>2-fold increase). They added that these genes had lower expression levels in normal conditions but exhibited a strong response to heat stress, suggesting their role in heat stress response. Six ZmJMJC genes, namely ZmJMJC2, ZmJMJC4, ZmJMJC7, ZmJMJC11, ZmJMJC12, and ZmJMJC16, showed significant downregulation (<0.5-fold decrease) in expression levels during heat stress compared to normal conditions (Zhou & Ma, 2008). The contrast behavior of JMJC family members in different plant system is yet to be answered.

Drought stress induced higher level of expression in nine TrJMJ genes of wheat with respect to time period as quick and late response. The study concluded that better candidate are available in JMJ family to tackle drought stress. In wheat the genes Tr-1A-JMJ2, Tr-1B-JMJ2, and Tr-1D-JMJ2 showed considerable increase in expression. This demonstrates their quick reaction to drought stress circumstances. Several genes were highly elevated following 24 hours of drought stress treatment, including Tr-4A-JMJ-C1, Tr-4B-JMJ-C1, Tr-4D-JMJ-C1, Tr-7A-JMJ-C1, Tr-7B-JMJ-C1, and Tr-7D-JMJ-C1. These genes may be important in the later phases of drought stress response (*Mali et al., 2023*). In *Oryza* species the expression of OsJMJ-C14 was found to be the most upregulated in the shoot. Under salt stress, two block duplicated genes, OsJMJ-C3 and OsJMJ-C13, were also elevated in the shoot. Under salinity stress, the expression of OsJMJ-C5 was downregulated in the shoot but increased in the root. Under salt stress, OsJMJ-C11 was significantly downregulated in the root (*Zhou & Ma, 2008*). In *Brassica napus* genes of AtJMJ15 (a/b/c), AtJMJ16 (a/b/c/d), and AtJMJ18 (a/b/c/d), showed considerable upregulation during drought, high-temperature, or salt stress. Genes, such as BnJMJ18;a, showed considerable upregulation in response to high temperature stress, although their counterparts (BnJMJ18;b/c/d) did not (*Xu et al., 2015*). In mung bean the expression levels of eight VrJMJ genes (VrJMJ3, VrJMJ5, VrJMJ10, VrJMJ11, VrJMJ14, VrJMJ15, VrJMJ17, and VrJMJ18) were comparatively high. In leaves, the expression of three VrJMJ genes (VrJMJ8, VrJMJ9, and VrJMJ13) was quite high. The expression levels of 12 VrJMJ genes were downregulated in response to short-day (SD) therapy as compared to their expression under long-day (LD) circumstances. Under SD circumstances, the expressions of VrJMJ3, VrJMJ4, VrJMJ7, VrJMJ8, VrJMJ12, and VrJMJ17 were elevated. Mostly expressed in leaves, VrJMJ8, VrJMJ9, and VrJMJ13 may play significant roles in the photoperiodic control of blooming in Mung bean (*Qian et al., 2019*). In *Taraxacum koksaghyz*, TkSJM23 exhibits a notable over expression in fully formed leaves than developing leaves, TKSJM25 gene has very low expression level and no substantial tissue- specific increase (*Shi et al., 2014*). In tea Upregulation of specific JMJ-C genes (e.g., TV1_JMJs_03.1, TV1_JMJs_05.4, and TV1_JMJs_09.1) was observed during cold, salinity, and dehydration stresses in the tea plant (TV1) (*Zhang et al., 2020*). In lichi Girdling Plus Defoliation (GPD) and ethephon (ETH) were used as two separate treatments to cause abscission in fruit-bearing shoots. These procedures were utilized to simulate the circumstances that cause litchi fruit to shed. The research suggests that the 17 HM genes may play a variety of roles in controlling the abscission

of litchi fruit. Some may participate in early signaling activities, whereas others may contribute in process's later phases (Wang et al., 2022).

In *Brassica napus*, under the drought treatment, majority of JMJC had significantly higher expression level. But BnJmJ14a, BnJmJ19a/c were exceptions. BnJmJ16;a, BnJmJ17;b/c and BnJmJ18; were highly expressed. Under high temperature only small portion of JMJC genes (6 out of 20) shows increased expression and BnJmJ18;a shows high expression. Under salt stress over half of the JMJC genes, including BnJmJ14, BnJmJ15;a, BnJmJ16;a, BnJmJ17;b/c, and BnJmJ18;a/d, was striking (Aiese Cigliano et al., 2013). In sweet orange, among the CsJMJS (Jumonji domain containing histone demethylases) family, CsJMJ11 was induced by the infection. Its expression levels went up following the pathogen attack. Three CsJMJs (CsJMJ1, 4, and 14) were strongly downregulated by the infection, indicating a decrease in their expression levels (Alves et al., 2022). Different expression patterns were seen in the genes in this cluster. C3: Throughout all tissues examined, the genes in this cluster showed modest expression levels. In the same 10 tissues, the study also looked at the expression patterns of recently duplicated JMJC gene pairs. Genes that have been duplicated, or paralogs, typically show identical expression patterns (Zhou & Ma, 2008). In *Citrus grandis*, Cg5g012890 showed extremely low expression (almost 0), and was essentially not expressed in these samples, whereas Cg2g045510 showed reasonably high expression levels in all three kinds (Zaidi et al., 2018). In potato, under high temperatures, gene expression analysis of StJMJs in heat-sensitive (HS) and heat-tolerant (HT) genotype leaf and stolon tissues was carried out. In the leaf tissue of both genotypes, the expression of StJMJ3, StJMJ6, StJMJ8, StJMJ10, StJMJ12, StJMJ15, StJMJ16, StJMJ17, StJMJ18, StJMJ19, and StJMJ21 was heat inducible; however, in the stolon tissue, the expression was antagonistic (upregulated in HS, downregulated in HT). Furthermore, it was shown that StJMJ11 was markedly elevated in the leaf and stolon of both genotypes (Takeuchi et al., 2006). In *Arabidopsis* and rice, 21 of these genes were shown to express under typical development conditions, proving that they are all actively expressed genes. The majority of these genes are widely expressed, with rosette leaves and inflorescences showing a slightly greater expression. Both At MEE27/JMJ15 and At JMJ16 are mostly expressed in siliques and inflorescences, respectively, suggesting that they may confer specialized function at the reproductive developmental stage (Ma et al., 2022). In *G. hirsutum* the study examined how the GhJMJC genes responded to abiotic stress situations, particularly osmotic stress (PEG treatment) and cold

stress (4°C). The majority of the JMJ-C gene family members in *G. hirsutum* were up-regulated in response to cold stress or osmotic stress, suggesting they may be involved in stress responses. In both cold and osmotic stress conditions, seven *G. hirsutum* JMJ-C genes (GhJMJ3A, GhJMJ18A, GhJMJ18D, GhJMJ19D, GhJMJ22D, and GhJMJ24A and GhJMJ24D) significantly increased in expression (Tsukada *et al.*, 2005). In bamboo, Under the influence of methyl jasmonate (MeJA) and abscisic acid (ABA), several JMJ-C genes were altered. The majority of JMJ-C genes were found to be strongly expressed in shoots, which suggests that they play key roles in the rapid growth of moso bamboo (Shi *et al.*, 2014).

2.6 RESPONSES OF JMJ-C GENES TOWARDS ABIOTIC STRESS:

Abiotic stress encompasses the detrimental effects of non-living factors on living organisms. These factors include extreme temperatures (both high and low), high salinity, drought, flooding, nutrient deficiencies, and toxic levels of metals. Throughout their life cycles, plants regularly encounter these abiotic stress conditions, posing significant challenges, particularly in agriculture. This has become an increasingly concerning issue for crop productivity due to environmental changes, pollution, and global warming. Unlike animals, plants are sessile organisms, and as such, they have evolved specialized mechanisms to adapt to adverse environmental conditions (Klose *et al.*, 2006). One key adaptation is the presence of the cuticle, an outer, transparent lipid structure that covers the aboveground parts of plant organs. Additionally, plants have evolved mechanisms to cope with the excessive production of reactive oxygen species (ROS) under abiotic stress. This overproduction triggers a series of responses, including the activation of oxygen antioxidants, the up-regulation of osmolytes, and the activation of stress-responsive genes. These responses are essential for enhancing a plant's tolerance to stressful conditions, enabling them to survive in adverse environments, such as extreme temperatures. The identification of specific genes that play a pivotal role in this regulatory process is crucial, as the overall mechanism relies on precise control of these genes. Ultimately, reducing oxidative stress contributes to an increase in stress tolerance factors and promotes the long-term sustainability of plants in challenging conditions (Li *et al.*, 2022). Many of the JMJ-C proteins are responsive to stresses and involved in such mechanisms.

CHAPTER 3

AIM AND SCOPE

AIM :

To understand the function and importance of JMJ-C genes in finger millet, with a special emphasis on their involvement in stress responses

SCOPE::

- 1)To identify and characterize the JMJ-C gene family members in *E. coracana*
- 2)To establish the evolutionary relationship of JMJ-C genes in *E. coracana* and with other plant species
- 3)To conduct the candidate profile studies to analyse the involvement of JMJ-C genes on abiotic stress
- 4)To understand their role in regulation of abiotic stress-responsive gene expression
- 5)To identify the candidate JMJ-C proteins which can serve as potential targets for crop improvement against abiotic stress

CHAPTER 4

MATERIALS AND METHODS

4.1 PLANT MATERIALS AND TREATMENT:

E.coracana seeds were purchased from a seed store and planted in 4-inch plastic pots at room temperature. A total of 9 pots were grown, divided into three batches, each containing 3 pots. Two batches were subjected to abiotic stress, while one group served as the control. The stresses applied included heat using an incubator maintained at 37-42 °C, salt using NaCl, and drought using PEG.

After the plants reached 30 days of age, they were treated with NaCl, PEG, and heat for a duration of 4 days. Following the stress treatment, plant tissue samples were collected for assays such as pigment, proline and weight analysis. These preliminary analyses were conducted before proceeding to expression analysis studies.

4.1.1 Pigment analysis:

Pigment analysis involved collecting 750 mg of leaf tissue from each stress-treated and control plant. The samples were ground using 80% chilled acetone and centrifuged at 5000 rpm for 5 minutes. The supernatant was collected, and optical density (OD) values were measured at 663 nm, 645 nm, and 470 nm.

4.1.2 Proline analysis:

For the Proline assay, 750 mg of leaf tissue from each stress-treated plant was ground and homogenized with 3% Salicylic acid. After centrifugation at 5000 rpm for 5 minutes, equal volumes of Glacial acetic acid and acid ninhydrin solution were added to the supernatant in a Falcon tube. The combined solution was placed in a boiling water bath for 30 minutes, and the reaction was terminated by cooling in ice. Toluene was added for layer separation, and the top layer was used for OD measurement at 520 nm.

Turgid weight determination involved weighing leaf samples from each stress-treated and control plant. The leaves were immersed in water in a centrifuge tube for 4 hours. After blotting, the weight was recorded, and turgid weight and water retention capacity were calculated.

4.2 INSILICO ANALYSIS

4.2.1 Identification and analysis of physico-chemical property of *jmj-c* gene members of *E.coracana* :

The HMM (Hidden Markov Model) files of the JumonjiC (JMJ-C) domain (PF02373) from the Pfam protein family database (<http://pfam.xfam.org/>) were employed to search predicted proteins of JMJ-C from the phytozome database (<http://www.phytozome.net/eucalyptus>), with the default setting. All Arabidopsis JMJ-C protein sequences were used as query sequence to perform Basic Local Alignment Search to search the *E.coracana* JMJ-C family members, with an E-value of $1e-5$. After filtering redundant sequences from the HMM and the protein blast outcomes, all retrieved sequences obtained by the combinatorial method were examined with the NCBI Conserved Domain Database (CDD) (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). InterPro database (<http://www.ebi.ac.uk/interpro/>) was used for accurate verification conserved protein domain (PF02373). The confirmed EcJMJ genes were named based on homology between the *E.coracana* and Arabidopsis genes. The protein signatures, such as amino acids number (AAs), isoelectric point (pI) and molecular weight (Mw) of the EcJMJ proteins were acquired using ProtParam in ExPASy (<https://web.expasy.org/protparam/>). The subcellular localization of EcJMJ genes was predicted with the CELLO Software (<http://pfam.xfam.org/>).

4.2.2 Classification and phylogenetic analysis of *Ecjmj* proteins:

MUSCLE was used to perform multiple sequence alignments of the full-length amino acid sequences of all predicted *E.coracana* JMJ-C proteins and the orthologs. The protein data of 21 Arabidopsis JMJ were extracted from the TAIR database (<https://www.arabidopsis.org>), and compared with EcJMJ proteins. The phylogenetic tree was constructed by the Neighbor Joining (NJ) method in MEGA7.0 software, with 1000 bootstrap replicates.

4.2.3 Gene structure and conserved motif analysis of *Ecjmj* genes:

The gene exon–intron structure information of the *E.coracana* JMJ-C gene family was extracted from the phytozome (<http://www.phytozome.net/eucalyptus>) database and was visualized using the Redraw Gene Structure (From GFF/GTF3 File) function in TBtools. The conserved motifs and domains of the EcJMJ proteins were analyzed by the online software MEME (<http://meme-suite.org/tools/meme>) and TBtool software, respectively. The visualized map of the motifs and conserved structural domains was performed by TBtool software.

4.2.4 Chromosome location of *Ecjmj* genes:

The chromosomal locations information of the EcJMJs were obtained from the NCBI (<https://www.ncbi.nlm.nih.gov/genome/>) database and then analyzed by TBtools software.

4.2.5 Cis-regulatory element analysis of *Ecjmj* genes:

The upstream sequences of EcJMJ (defined as 2kb away from the transcription start site) were downloaded and submitted to PLANTCARE(<http://bioinformatics.psb.ugent/Plantcare/html>) for the promoter cis-element analysis, and visualization was performed using TB tool software.

4.2.6 Interaction and structural – functional relationship of *Ecjmj* proteins:

The peptide sequences of all the 16 EcJMJs were submitted to the STRING(<https://string-db.org/>) database for prediction of the interacting networks and to the I-TASSER(<https://zhanggroup.org/I-TASSER/>) for predicting the structural functional relationships of Ec-JMJs.

4.3 GENE EXPRESSION ANALYSIS

4.3.1 Rna extraction and quantitative Rt-pcr:

Different tissue samples of *E.coracana* were extracted, and 1 µg of RNA was extracted from each sample. First-strand cDNA was synthesized by the PrimeScript® RT reagent Kit with gDNA Eraser according to the manufacturer's instructions. The obtained cDNA was diluted tenfold as the template for quantitative real-time PCR. The EcJMJ gene-specific primers were designed by Primer BLAST of NCBI (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). RT-qPCR was performed using a 96-well Chromo4 Real-Time PCR system with SYBR® Premix Ex Taq™II

(Takara RR820A). The RT-q PCR conditions were as follows: pre denaturation at 95 °C for 30 s, followed by 40 cycles at 60 °C for 30 s. The relative gene expression values of EcJMJ were calculated with the $2^{-\Delta\Delta C_t}$ method. Three independent biological replicates and three technical replicates were used for each sample. The data analyses with the mean $\pm$ standard deviation of three replications were visualized in GraphPad Prism 8.

4.4 STATISTICAL ANALYSIS

Each treatment consisted of 30 plants and each experiment was repeated thrice. Pigment and proline analyses were studied after the stress treatment. Data were analyzed using GraphPad Prism. The mean values of treatments were subjected to t-test. Significance was determined at $p < 0.05$ level (Gomez and Gomez, 1976) using the software GraphPad Prism for windows OS.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 IDENTIFICATION OF JMJ-C GENES IN *E. CORACANA*:

We performed genome-wide identification of EcJMJ proteins by a combinatorial approach of the domain characteristics, CDD, and InterPro database. A total of 16 JMJ-C genes were identified and then designated EcJMJ1 to EcJMJ16 based on homology between the *E. coracana* and *Arabidopsis* genes. The basic information of EcJMJs, gene ID, molecular weight (Mw), isoelectric point (pI) and protein length are listed in Table 5.1. The protein length of EcJMJ showed various differences, which varied from 514 aa (EcJMJ11) to 1872 aa (EcJMJ7 and EcJMJ8); the corresponding coding sequence length of the EcJMJ genes varied from 1542 bp (EcJMJ11) to 5616 bp (EcJMJ7 and EcJMJ8); the molecular weights ranged from, and the predicted pI was between a minimum of 5.57 (EcJMJ16) and a maximum of 9.14 (EcJMJ14) (Table 5.1). The prediction of subcellular localization found that many *E. coracana* JMJ-C gene family members were located in the nucleus, which was consistent with the occurrence of silencing redundant parts of the genome by histone demethylation to ensure the structural and functional integrity of the genome. The number of JMJ-C genes tends to be conserved in different species, with 21, 21, 20 and 20 JMJ-C genes in *Arabidopsis thaliana*, *Vitis vinifera*, *rice* (ChromDB database) and *Solanum lycopersicum*, respectively. The reduced JMJ-C gene number in *E. coracana* implied that several essential EcJMJ genes may undertake a comprehensive role in regulating plant growth during *E. coracana* evolution (Shi et al., 2023).

The JMJ-C gene family in *E. Coracana* (finger millet) comprises 16 identified members, distributed across four subfamilies: KDM5, KDM4, JARID1, and KDM3. This distribution pattern is comparable to findings in other plant species such as wheat, which has 24 JMJ-C genes divided into two subfamilies (Wang et al., 2022), maize with 19 JHJDM proteins grouped into three subfamilies (Qian et al., 2019), and mungbean, where 18 vrJmj genes are identified and

categorized into four subfamilies (*Li et al., 2023*). Similarly, moso bamboo harbors 22 JMJ-C genes distributed across four subfamilies (*Shi et al., 2023*), 17 JMJ-C proteins were identified in melon (*Cucumis melo* L.) and were categorized into five subfamilies (*Jin et al., 2023*). While *E. Coracana* demonstrates a moderate number of JMJ-C genes, other species like *Brassica napus* boast a higher diversity, with 65 genes grouped into seven subfamilies (He et al., 2021), while some, like *Rosa chinensis*, have fewer members, with only 12 JMJ-C genes (Dongetal.,2020).

Table 5.1-Basic information of EcJMJs

GENE NAME	GENE ID	CHROMOSOME	LOCATION	CDS LENGTH/BP	PROTEIN			
					AA	Mw (Da)	PI	SUBCELLULAR LOCALIZATION
EcJMJ1	2AG0149820.1	2A	60,181,508-60,186,786(-)	2517	839	95191.4	7.44	Nuclear
EcJMJ2	6AG0516680.1	6A	9,791,479-9,796,998(+)	2346	782	88433.27	5.68	Nuclear
EcJMJ3	6BG0468390.1	6B	9,196,435-9,202,176(+)	2697	899	101135.16	6.24	Nuclear
EcJMJ4	2BG0204370.1	2B	69,513,006-69,518,297(+)	2874	958	108438.6	8.55	Nuclear
EcJMJ5	5AG0399940.1	5A	57,127,311-57,132,741(-)	2937	979	109970.93	5.68	Nuclear
EcJMJ6	5BG0448630.1	5B	73,228,510-73,233,927(-)	3048	1016	114047.55	5.76	Nuclear
EcJMJ7	6BG0505330.1	6B	74,165,174-74,182,806(+)	5616	1872	211204.33	6.05	Nuclear
EcJMJ8	6AG0551750.1	6A	59,498,035-59,514,139(+)	5616	1872	211379.65	6.27	Nuclear
EcJMJ9	2BG0172050.1	2B	16,180,320-16,185,429(+)	2517	839	94776.17	8.33	Nuclear
EcJMJ10	2AG0141850.1	2A	54,661,700-54,667,041(+)	2361	787	89005.53	6.14	Nuclear
EcJMJ11	2BG0197400.1	2B	64,778,889-64,782,080(+)	1542	514	58872.75	6.97	Nuclear
EcJMJ12	1BG0093660.1	1B	69,845,423-69,851,938(+)	3522	1174	130565.92	9.02	Nuclear
EcJMJ13	1AG0043650.1	1A	54,483,353-54,489,831(+)	3531	1177	130493.67	9.01	Nuclear

EcJMJ14	2AG011 8360.1	2A	17,508,818- 17,515,663(+)	2544	848	94634.9	9.14	Nuclear
EcJMJ15	5AG038 6710.1	5A	28,294,196- 28,303,296(-)	4233	1411	156282.6 7	7.62	Nuclear
EcJMJ16	3BG029 4000.1	3B	61,732,235- 61,740,142(-)	2595	865	94722.55	5.57	Nuclear

5.2 PHYLOGENETIC ANALYSIS OF THE JMJ-C GENES:

To explore the evolutionary relationship of EcJMJ genes, we constructed a phylogenetic tree using 32 JMJ-C protein sequences referenced *Arabidopsis thaliana* (Fig. 1). According to the JMJ-C subfamilies in *Arabidopsis*, the phylogenetic tree was branched into four groups, KDM4/JHDM3, KDM5/JARID1, JARID1, KDM3/JHDM2, by blasting the sequence similarities and common conserved domains. Among them, JARID1 is the largest subfamily, KDM3/JHDM2 subfamily has a minimum of members (Fig. 1). In *E. coracana*, the member of subfamilies showed that there are six gene members in KDM5 subfamily, four gene members in KDM4 and JARID1 subfamily and two in KDM3 subfamily (Fig. 2A) with respect to *Arabidopsis* members (He et al., 2021).

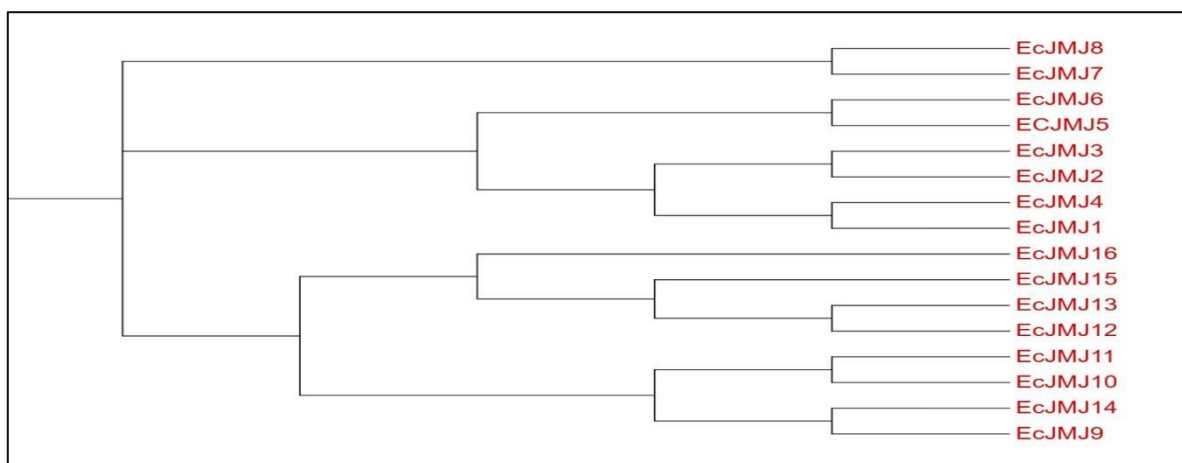


Fig 5.1 Phylogenetic relationship of JMJ-C gene family members in *E. coracana*

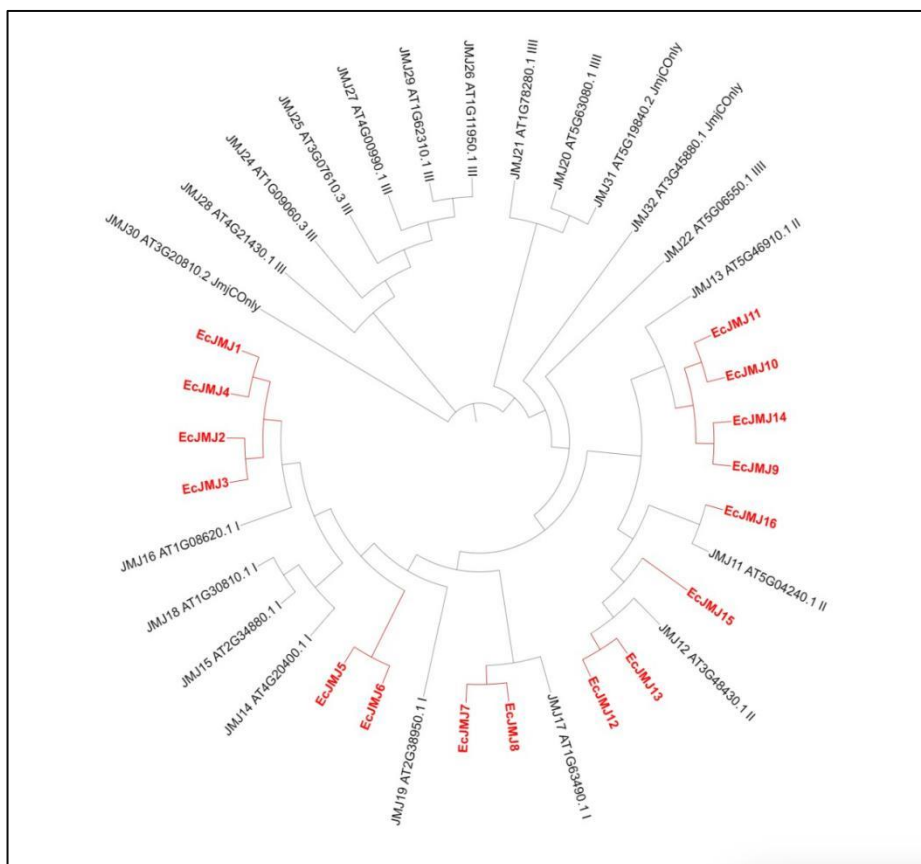


Fig 5.2-Phylogenetic relationship of JMJ-C members with Arabidopsis

5.3 CONSERVED MOTIF AND GENE STRUCTURE ANALYSIS OF EcJMJ GENES:

We analyzed the conserved motif and gene structure of all 16 EcJMJs and identified 10 motifs in *E.coracana* (Fig. 2B). KDM3 subfamily genes have motif1, motif2, motif3, motif4, motif5, motif7, and motif8 and align in the order of 3-4-5-1-7-2-8. In the KDM4 and JARID1 subfamilies, most EcJMJs contain motif1, motif2, motif3 and motif5, and align in the order of 3-5-1-2 (Fig. 2B). The KDM5 subfamily contains all the 10 motifs and align in the order 6-8-3-4-5-1-7-2-9-10 (Fig. 2B). The regular motif alignments in the different subfamilies indicated the sequence conservation within subfamilies and validated the reliability of the phylogenetic tree division. The conserved domain analysis showed that all *E.coracana* JMJ-C proteins have JMJ-C domain. FYRC and FYRN superfamily domains appeared in the EcJMJ1-EcJMJ6 proteins, and the longest protein EcJMJ7 and EcJMJ8 had the most domains, containing JMJN superfamily, ARID superfamily, PHD_SF superfamily, JMJ-C, zf-C5HC2 superfamily, PLU-1superfamily, ARID superfamily (Fig. 2C). The gene structure analyses in EcJMJs exhibit tremendous variation in the intron number,

which ranges from 8 to 33 (Fig. 2D). We assumed that the evolutionary diversification of JMJ-C family protein structures and functions may be conferred by the loss or gain of their conserved domains.

The comparative study of JMJ-C gene families across different species reveals notable similarities and differences in conserved motifs and gene structures. In wheat, analysis of 24 JMJ-C family proteins identified ten conserved motifs, with seven relatively conserved motifs present in all proteins, indicating essential roles in maintaining conservative core functions. Proteins within the same subfamily exhibit similar motif composition and distribution patterns. Additionally, JMJ-C family proteins display comparable secondary and tertiary structures, suggesting functional similarities within subfamilies (Wang *et al.*, 2022).

In melon, 17 CmJMJ genes were analyzed, revealing ten conserved motifs and diverse gene structures with exon numbers ranging from 2 to 35. Similarly, in finger millet (*E.coracana*), 16 EcJMJ genes exhibited ten conserved motifs, with variations in gene structure characterized by intron numbers ranging from 8 to 33 (Jin *et al.*, 2023).

The structural analysis of BpJMJ genes in *Brassica napus* highlights significant heterogeneity in exon-intron organization, with an average exon count of 13.52 per gene and notable variability across different subfamilies. Notably, transposon insertions contribute to variations in gene structure, particularly in genes such as BpJMJ08, BpJMJ09, BpJMJ10, BpJMJ14, and BpJMJ17, resulting in unusually long introns JMJ-C gene expression profiles across various plant species reveal diverse responses to different stress conditions (Dong *et al.*, 2020).

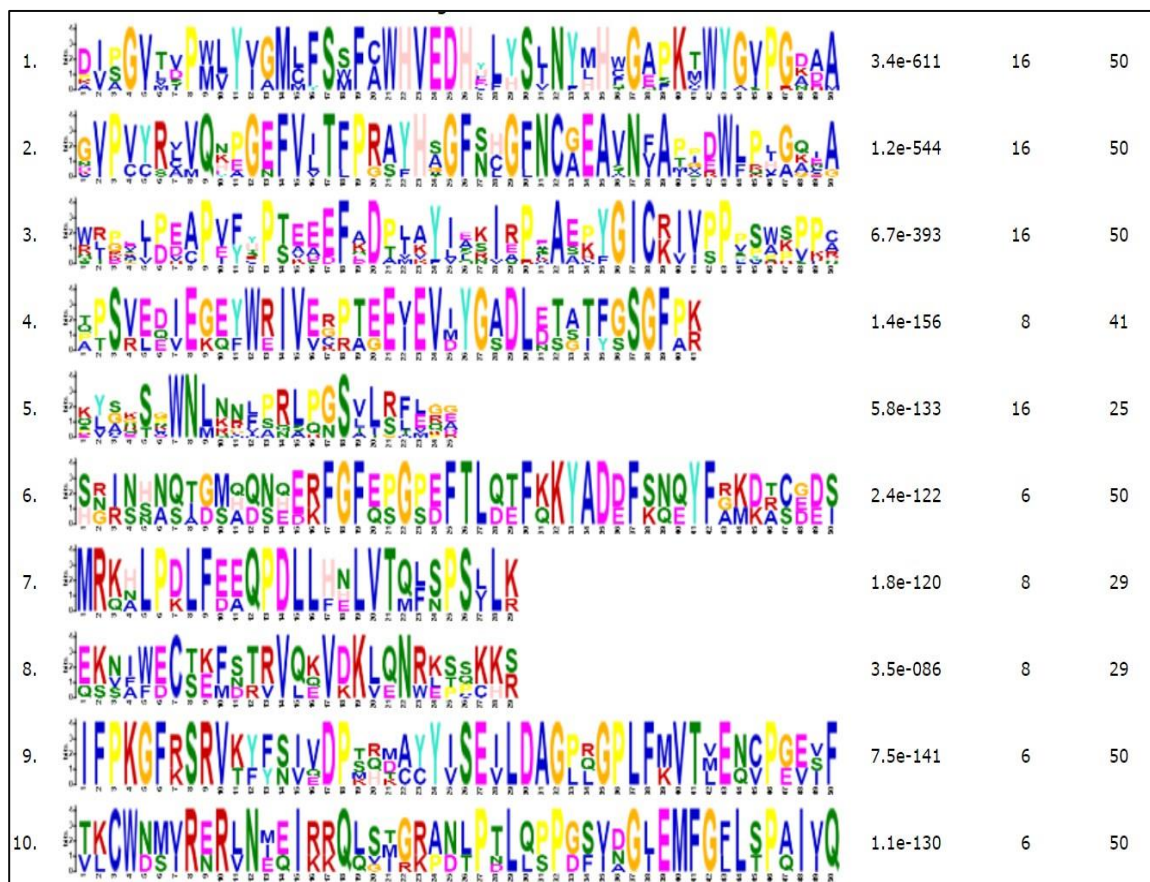


Fig 5.3a Motif analysis. Ten motifs in EcJMJ proteins are indicated by different colored sequences

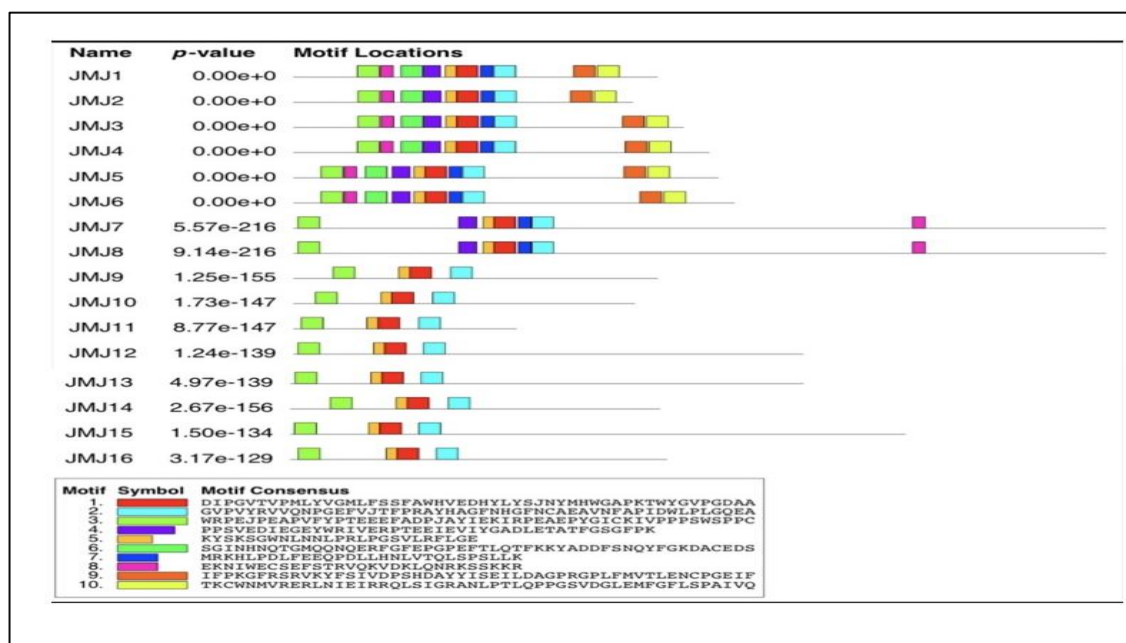


Fig 5.3b Motif analysis. Ten motifs in EcJMJ proteins are indicated by different colored squares

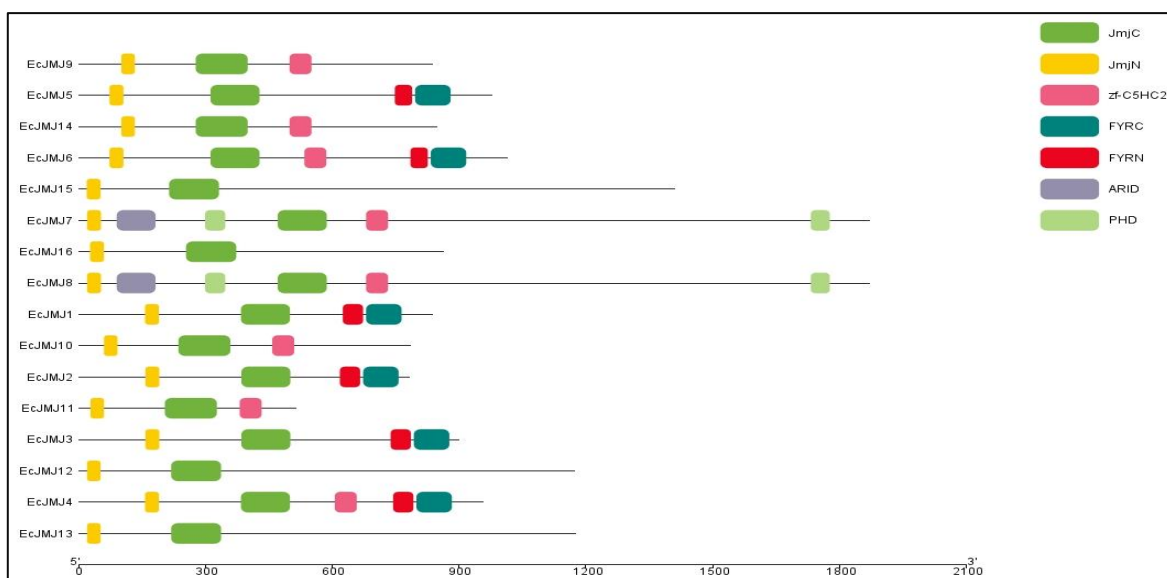


Fig 5.4 Schematic structure of conserved domains of EcJMJ proteins. The figure displays the position and size of the different conserved domain, which is indicated by colored shapes and generated from Tbtools.

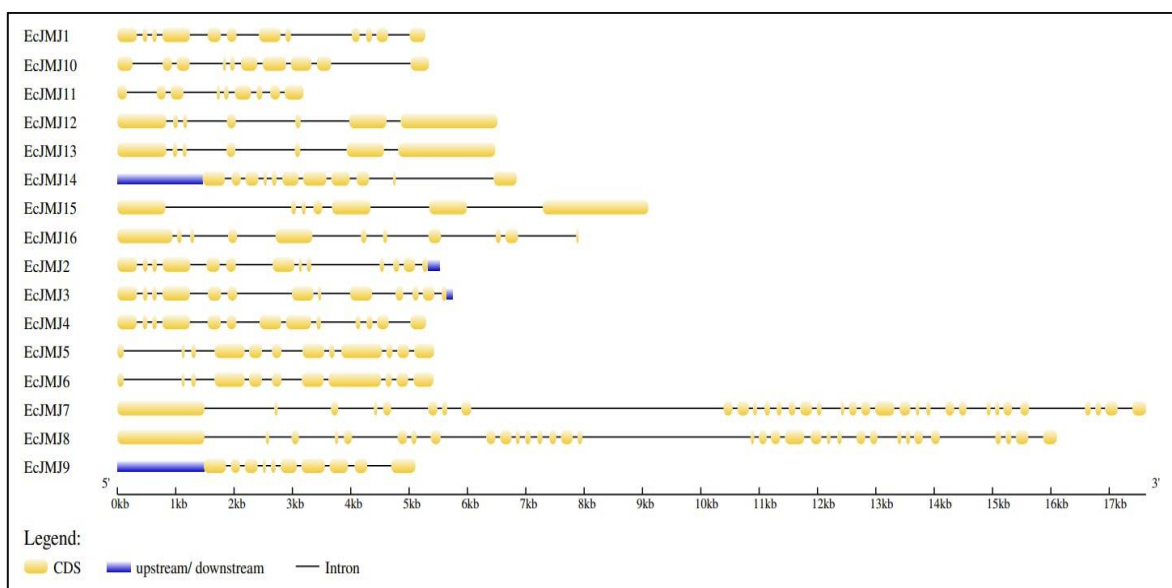


Fig 5.5 Gene structure analysis of EcJMJ genes

5.4 CHROMOSOME DISTRIBUTION OF EcJMJ GENES:

Chromosomal localization analysis revealed that EcJMJ genes were evenly distributed on nine chromosomes, which displayed Three genes on chr2A,Chr2B and two genes on Chr5A,Chr6A and Chr6B(Fig.3) and one gene on Chr1A,Chr1B, and ,Chr 3B and Chr 5B (Fig. 3).*E.coracana* (finger millet) exhibits a uniform distribution of JMJ-C genes across its nine chromosomes, with varying gene counts per chromosome, such as three on certain chromosomes and two or one on others. In contrast, *Brassica napus* (He et al., 2021) displays a more intricate genomic landscape, with its 65 JMJ-C genes dispersed across 19 chromosomes, reflecting the repercussions of polyploidization events, tandem duplications, and subgenome-specific divergence. Similarly, Mung bean's JMJ-C genes are unevenly distributed over its 11 chromosomes, featuring varying gene counts per chromosome. Additionally, wheat showcases a diverse pattern of JMJ-C gene distribution across its 21 chromosomes, with some chromosomes bearing multiple genes while others contain only one and melon shows uniform gene distribution across its chromosome.

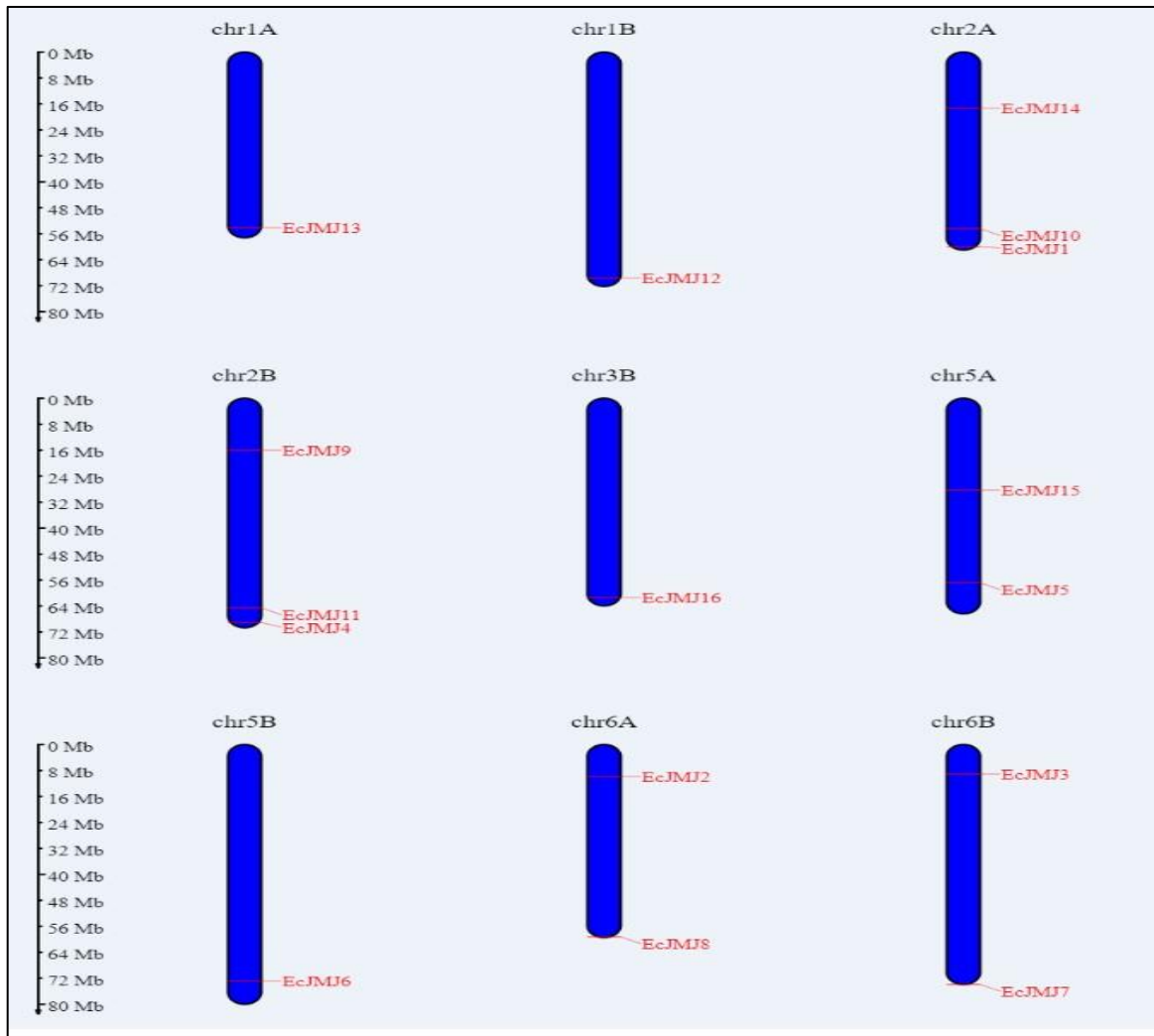


Fig 5.6 16 EcJMJ genes are mapped on the chromosomes. EcJMJs are distributed among nine chromosomes were indicated and red lines indicated the gene location

5.5 CIS-ELEMENT ANALYSIS OF THE PROMOTER REGION OF THE EcJMJ GENES:

To further clarify the possible regulatory mechanisms of EcJMJ genes, we analyzed the cis-acting elements in the promoter regions (2000-bp upstream sequence from the translation start site) of EcJMJ genes by PlantCARE database (Fig. 4). We found the cis-acting elements involved in phytohormone (abscisic acid, gibberellin, MeJA, auxin and salicylic acid), light response, growth and development, and abiotic stress in EcJMJ genes. Light-responsive elements were found in all EcJMJ, and gibberellin-responsive, anaerobic induction, auxin-responsive element, MeJA-

responsive and abscisic acid-responsive elements had the most common distribution. The hormone-related cis-acting elements were commonly presented in all subfamilies of EcJMJ: Auxin-responsive elements were present in the KDM4 and KDM3 subfamily genes, and MeJA-responsive elements were presented in all the EcJMJs. We also found that abiotic stress-related elements (low-temperature responsive, drought-inducible, defense and stress-responsive and anaerobic-inducible elements) were enriched in EcJMJs.

In finger millet (*E.coracana*), the promoter regions of EcJMJ genes contain elements associated with phytohormone responses (e.g., abscisic acid, gibberellin, MeJA, auxin, and salicylic acid), light response, growth and development, and abiotic stress. Similar analyses in other species demonstrate distinct patterns: birch suppresses gene expression in response to low temperatures, while potato and grapes contain elements responsive to light and stress. Rice species exhibit elements related to anaerobic conditions, ABA hormone, MYB transcription factors, and heat shock. In Tormentosa, promoters of CgJMJ-C genes are enriched with ABRE elements, indicating involvement in abiotic stress responses (Tian et al., 2021). Furthermore, Moso bamboo and *Gossypium hirsutum* genes respond to hormone treatments like ABA and MeJA, implying their role in hormone-mediated reactions.

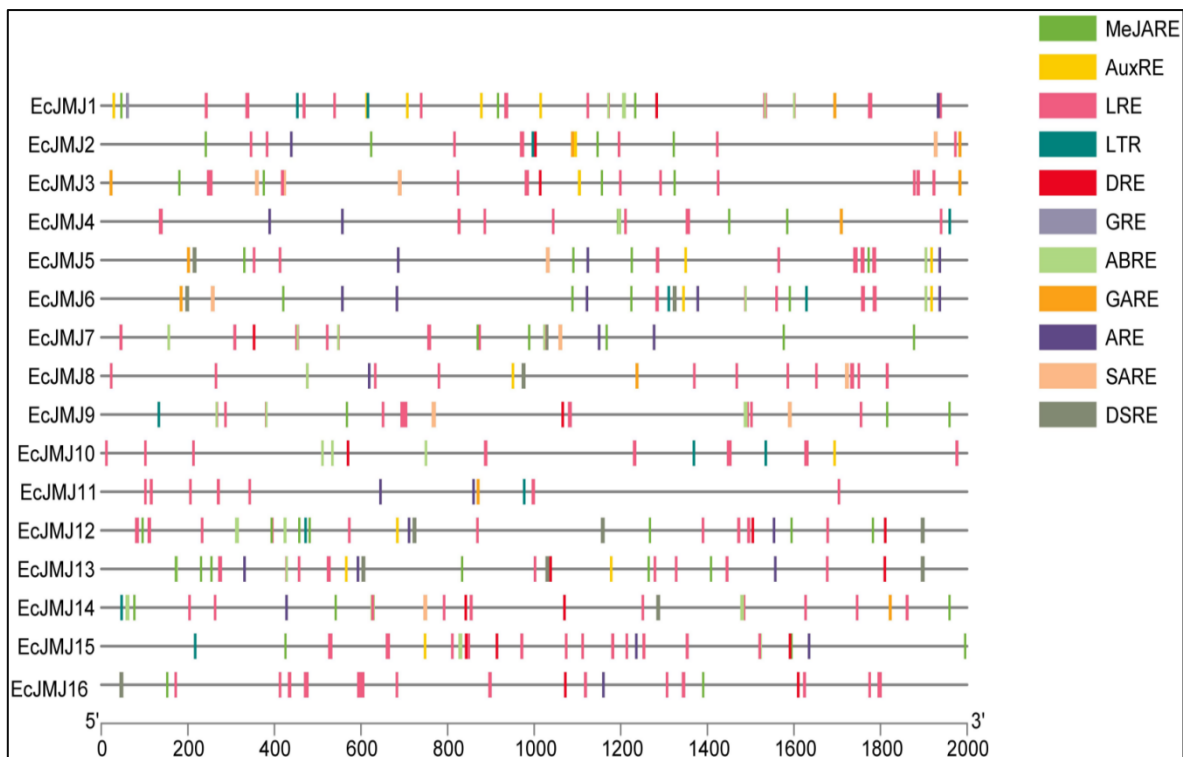


Fig 5.7 Cis-acting elements of the EcJMJ genes. The different colored lines indicate the position and number of the cis-acting elements located in the 2000bp upstream promoter region

5.6 INTERACTING NETWORK OF EcJMJ GENES:

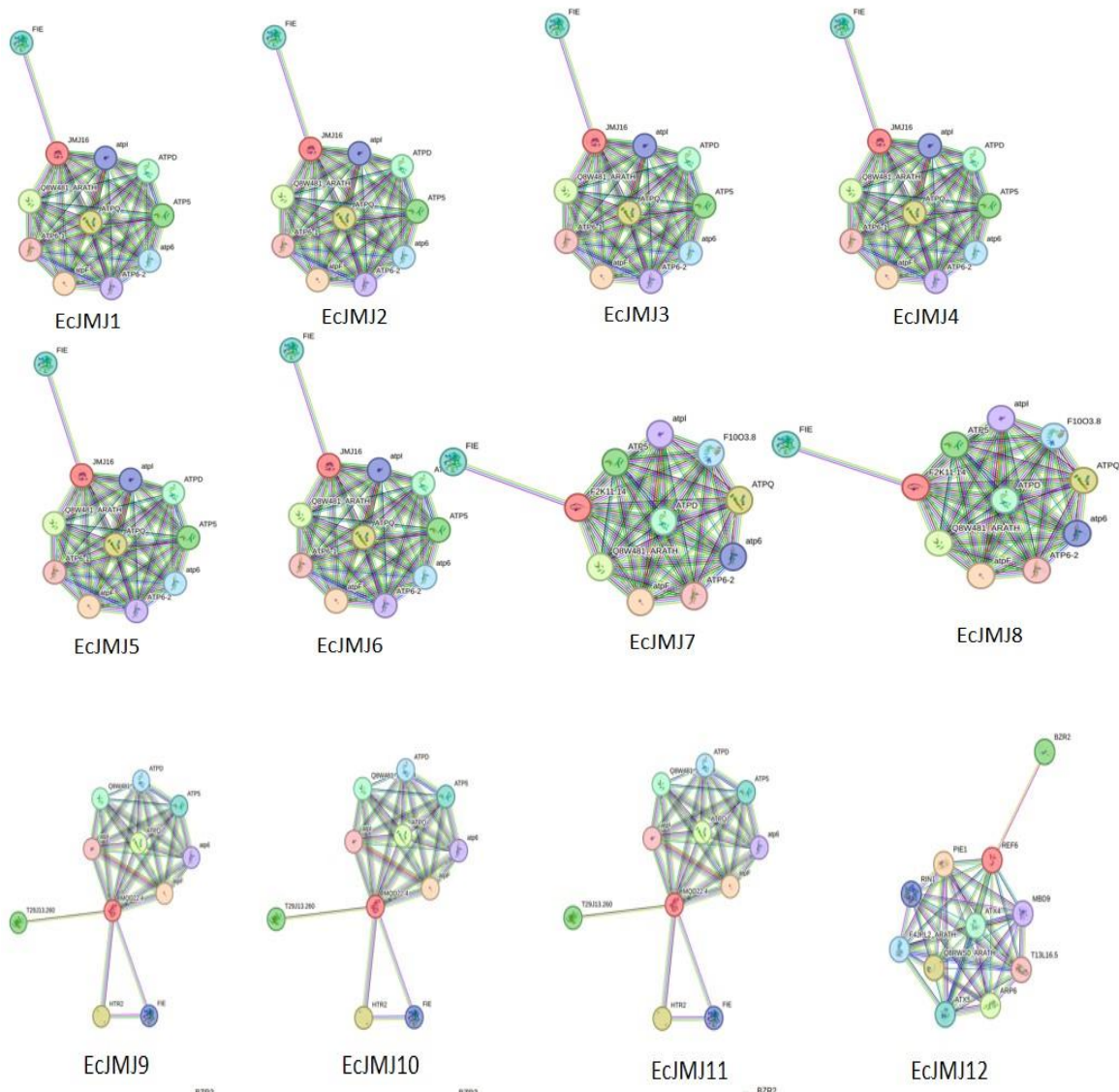
The EcJMJ1, EcJMJ2, EcJMJ3, EcJMJ4, EcJMJ5, EcJMJ6 of KDM5 subfamily interacts mainly with ATP synthase protein (atpl, ATPD, ATP5, atp6, ATP6-1, ATP6-2, atpf), FIE protein, and ARATH protein. A protein called ARATH is present in plants, especially in *Arabidopsis thaliana*. Although its exact role is unknown, it is thought to be involved in plant development and stress response. Plants include a protein called FIE (FERTILIZATION-INDEPENDENT ENDOSPERM), which is present in the endosperm tissue. It has been connected to the regulation of gene expression in the endosperm and plays a role in the formation of seeds. The ATP synthase complex, which is present in the inner membrane of mitochondria and chloroplasts, is made up of the proteins ATP6, ATP5, and ATPQ. The primary energy unit of the cell, ATP, is made by ATP synthase using a proton gradient that the electron transport chain creates. This complex's important elements are ATP6, ATP5, and ATPQ, each of which has a distinct function in the synthesis of ATP. The EcJMJ7, EcJMJ8 of KDM3 subfamily interacts with ATP synthase protein (ATP5, atpl, ATPQ, atp6, ATP6-2, atpf), ARATH protein, FIE protein, F2K11.14 protein. A protein in *Arabidopsis thaliana* is called F2K11.14. It is a member of the DUF6 domain-containing proteins family of proteins, which is present in many different types of organisms, including plants. It is believed that proteins containing DUF6 domains are involved in a number of biological functions. The EcJMJ9, EcJMJ10, EcJMJ11 of JARID1 subfamily interacts with ATP synthase protein (ATPD, ATP5, atp6, atpf, ATPQ, atp1), FIE, HTR2, Q8W481_ARATH, MQD22.4, T29J13.260 proteins.

A fundamental part of the chromatin structure, the nucleosome is made up of histone H3.2. DNA is encircled by a core of histone proteins, such as histone H3.2, to form nucleosomes. Histones, such as H3.2, compact DNA into chromatin and control its accessibility to cellular machinery, which is essential for controlling gene expression, DNA repair, DNA replication, and chromosomal integrity. The intricate system of post-translational changes of histones, referred to as the histone

code, and nucleosome remodeling activities further control the accessibility of DNA. MQD22.4 is a transcription factor jumonji (Jmj) family protein / zinc finger (C5HC2 type) family protein. The EcJMJ12, EcJMJ13 of KDM4 subfamily interacts with F4JPL2_ARATH protein, Q8RWS0_ARATH protein, RIN1, PIE, REF6, B2R2, HBD9, ARP6, ATX4, ATX5 proteins. Plant defense responses are mediated by RIN1 (RPM1-interacting protein 1). It engages in interactions with the RPM1 protein, a resistance protein that offers immunity against specific infections. Pleiotropic Regulatory Locus 1 (PIE) is involved in the regulation of several aspects of plant growth, such as hormone responses, blooming time, and leaf morphology. The histone demethylase known as REF6 (Relative of Early Flowering 6) regulates chromatin structure and gene expression, which in turn affects flowering time and other developmental events. B2R2 is a transcriptional regulation-related component of the Mediator complex. It most likely mediates connections between RNA polymerase II and transcription factors. The transcription factor HBD9 (Homeobox Domain Protein 9) is involved in several facets of plant growth and development. It controls gene expression in response to environmental cues like light and temperature. Actin-Related Protein 6 (ARP6):

ARP6 is a part of the SWR1 chromatin remodeling complex, which is responsible for switching out histone H2A for the variant H2A.Z. Controlling gene expression and other chromatin-related functions depends on this mechanism. ARX4 is also known as Arabidopsis Trithorax 4, ATX4 is a histone methyltransferase that modifies histone H3 at lysine 4 (H3K4) to add methyl groups, a change linked to active gene expression. Like ATX4, ATX5 (Arabidopsis Trithorax 5) is a histone methyltransferase that modifies histone H3 at lysine 4 (H3K4) by adding methyl groups. The EcJMJ 14 of JARID1 subfamily interacts with ATP synthase protein (ATPD, ATP5, atp6, atpf, ATPQ, atp1), FIE, HTR2, Q8W481_ARATH, MQD22.4, T29J13.260 proteins. The EcJMJ15 of KDM4 subfamily interacts with F4JPL2_ARATH, Q8RWS0_ARATH, RIN1, PIE, REF6, B2R2, HBD9, ARP6, ATX4, ATX5 proteins. The EcJMJ16 of KDM4 subfamily interacts with F10A5.19, T6J4.12, MGH3, FIE, CLF, EZA1, HTR2, HTR4, HTR11, HTR12 proteins. Essential parts of nucleosomes, known as HTR proteins, wrap and compact DNA into chromatin, limiting its accessibility to cellular machinery that uses DNA as a template. These proteins are essential for the control of transcription, chromosomal stability, DNA replication, and repair. H3.3 is mostly incorporated into chromatin in non-dividing cells and is not dependent on DNA synthesis. HTR12

is a variation that only takes the place of standard H3 in centromeric chromatin. It is necessary for chromosomal segregation and mitotic advancement because it recruits and assembles kinetochore proteins (*Xu et al., 2015*). These proteins play a role in the intricate processes of nucleosome remodeling and histone coding that control DNA accessibility. Histone lysine methyltransferase CLF modifies histones to control gene expression. Early Flowering 6 (ELF6): In response to environmental stimuli, ELF6 controls the timing of flowering. Mannan Gluco-oligosaccharide Hetero-syndrome 3 (MGH3): The Golgi apparatus is involved in the manufacture of mannan.



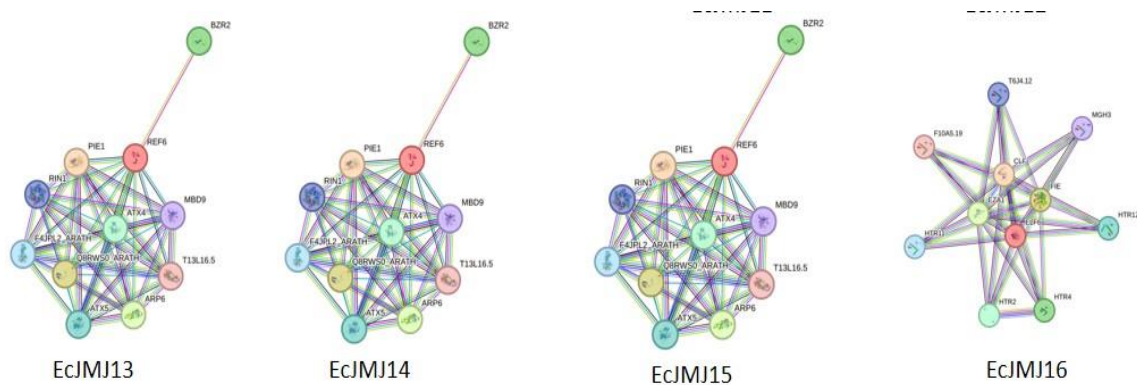


Fig 5.8 Interacting network of EcJMJ proteins using STRING tool.

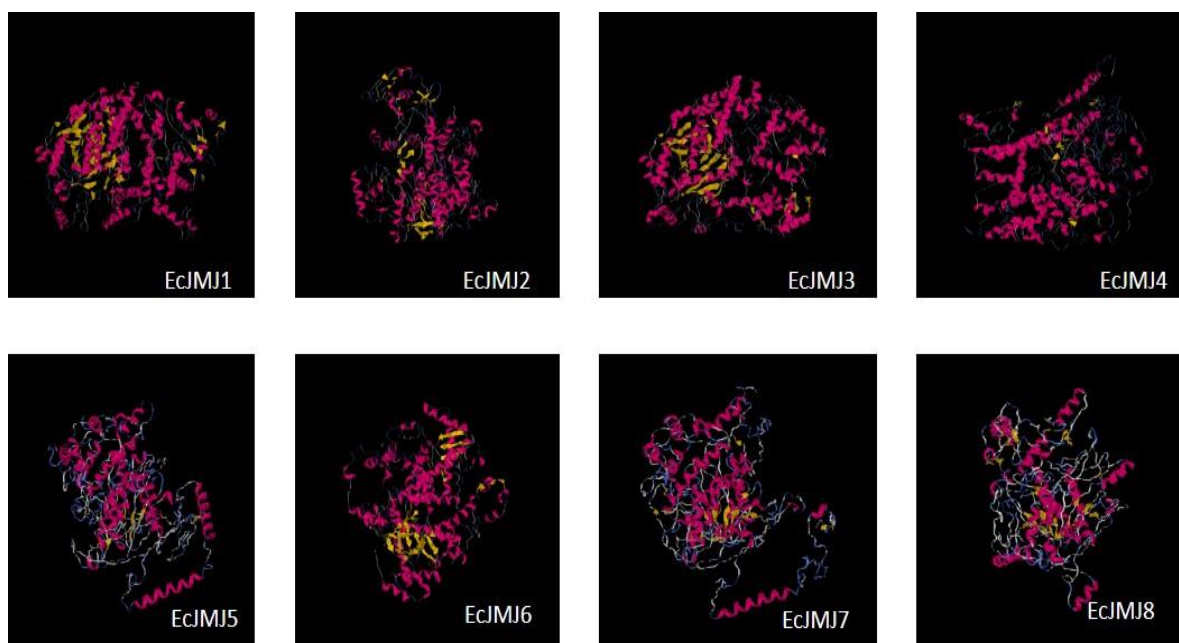
5.7 STRUCTURAL-FUNCTIONAL RELATIONSHIP OF EcJMJ PROTEINS:

The structural features of a protein predict its putative interactions and binding to various other molecules or ligands, and eventually provide its sequence–structure–function relationships. The sequence identity/similarity and accurate alignment between the template and a target protein leads to the prediction of 3D models.

The predicted normalized B-factor indicates how much each part of the protein moves naturally. The top 10 threading templates used by I-TASSER are the best-matching structures found in databases for building the protein model. I-TASSER generates many structural models, called decoys, and selects the best ones based on their similarity to known structures and how well they fit the data. The confidence of each model is measured by a C-score. TM-score and RMSD show how closely the predicted model matches known structures in the database.

The I-TASSER analysis for EcJMJ12 yielded several predicted models with varying TM-scores and RMSD values. The top predicted model displayed a TM-score of 0.553 and an RMSD of 2.31, aligning closely with PDB entry 8jchM. Following closely, the second-best model showed a TM-score of 0.479 and an RMSD of 2.81, aligning with PDB entry 7opkA. Additionally, the third-best model had a TM-score of 0.479 and an RMSD of 3.21, aligning with PDB entry 3fqdA. Subsequent models showed decreasing TM-scores and increasing RMSD values, each aligning with different PDB structures.

The I-TASSER analysis for EcJMJ13 yielded multiple predicted models with varying TM-scores and RMSD values. The top predicted model exhibited a TM-score of 0.29 and an RMSD of 0.3603, aligning closely with the structure of PDB entry 3gb8A. Similarly, the second-best model had a TM-score of 0.29 and an RMSD of 0.3742, aligning with PDB entry 3gjaA. Other models showed TM-scores ranging from 0.13 to 0.21, with corresponding RMSD values between 0.3124 and 0.3995, aligning with various PDB structures (*Xu et al., 2015*).



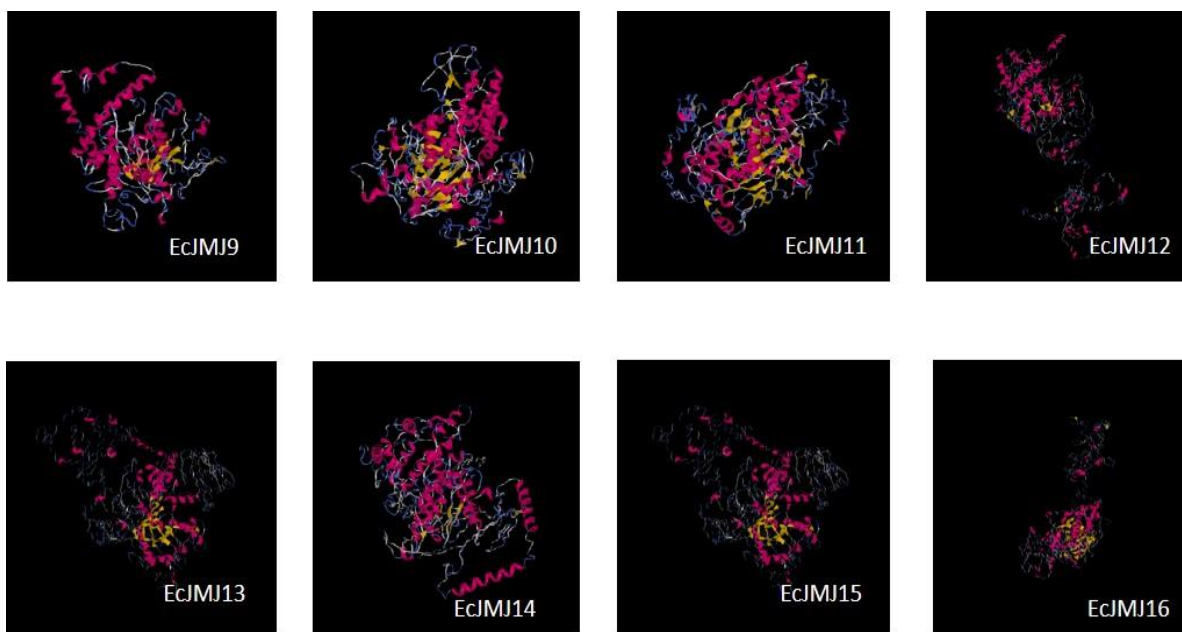


Fig 5.9 Predicted protein structure of EcJMJs generated using iTASSER tool



Fig 5.10 *Abiotic stress treatments were given to thirty-day old plants*

Table 5.2 Physical parameters of *E. coracana* during abiotic stress conditions

Treatment	ROOT LENGTH (cm)	SHOOT LENGTH (cm)
Control 1	4.5	11.8
Control 2	5	10.5
Control 3	5.5	11.0
D1H1	4.6	9.8
D2H2	4.7	7.6
D3H3	5	6
D4H4	6	4
D1S1	5.2	9.7
D2S2	5.1	9.1
D3S3	5.5	8.5
D4S4	6	8.1
D1Dr1	5.4	8.9
D2Dr2	5.9	8.7
D3Dr3	6.1	7.5
D4Dr4	6	7.2

5.8 PIGMENT, PROLINE AND RWC ANALYSIS :

The examination of pigment levels in plants subjected to heat, salinity, and drought stress indicated a significant decrease in chlorophyll A, chlorophyll B, and carotenoid concentrations compared to non-stressed plants. Conversely, the proline content in stress-treated plants exhibited a remarkable increase in contrast to control plants, suggesting that the plant is employing proline as an energy source for survival. Weight analysis further demonstrated a reduction in relative water content across all three stress conditions when compared to the control, indicating a decline in water retention within the plants under stress.

Table 5.3- Estimation of chlorophyll pigment in control and stresses *E.coracana* plants

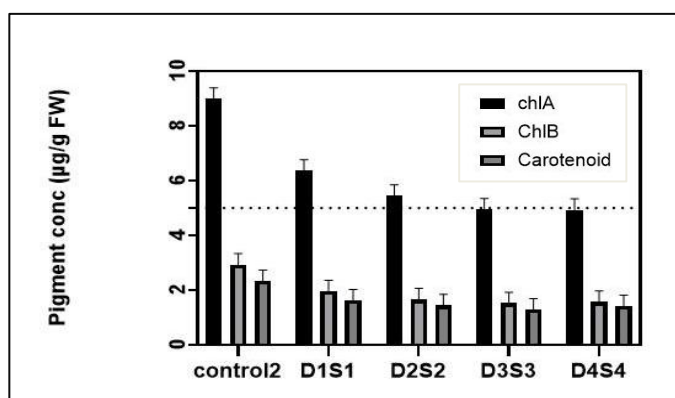
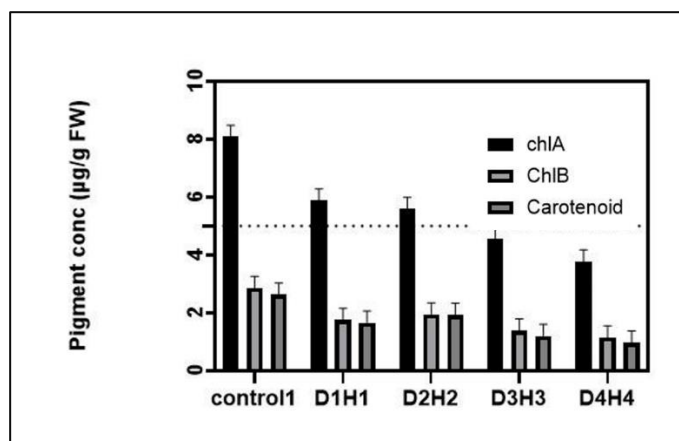
Treatments	663nm	645nm	470nm	chlA	chlB	Carotenoid
Control1	1.445	0.556	1.579	8.0908128	2.865504	2.629583845
Control2	1.602	0.595	1.449	8.997528	2.9415072	2.334311968
Control3	1.533	0.559	1.33	8.6233872	2.7007968	2.139916835
D1H1	1.043	0.373	0.994	5.8765104	1.7570208	1.660199786
D2H2	0.997	0.381	1.144	5.5857648	1.9482912	1.934907803
D3H3	0.809	0.292	0.734	4.5546336	1.3923264	1.203782154
D4H4	0.671	0.242	0.601	3.7779456	1.1527296	0.982558406
D1S1	1.132	0.41	1.001	6.37128	1.9637952	1.626406037
D2S2	0.968	0.349	0.88	5.4502992	1.6616928	1.444949807
D3S3	0.88	0.318	0.794	4.9538784	1.518624	1.299278089
D4S4	0.878	0.322	0.855	4.9365216	1.5670848	1.416693286
D1Dr1	1.031	0.465	1.394	5.684568	2.7952416	2.273620268
D2Dr2	0.936	0.433	1.391	5.1467664	2.6569056	2.30117427
D3Dr3	0.815	0.303	0.771	4.5770064	1.49976	1.257763806
D4Dr4	0.645	0.239	0.68	3.6233232	1.17816	1.143664125

Table 5.4 -Estimation of proline content in control and stressed *E.coracana* plants

Treatment	WEIGHT(grams)	A520	PROLINE CONTENT
Control1	0.151	0.002	0.033160
Control2	0.155	0.002	0.034038
Control3	0.154	0.002	0.033818
D1H1	0.149	0.035	0.572607
D2H2	0.156	0.04	0.685152
D3H3	0.15	0.055	0.905850
D4H4	0.15	0.07	1.152900
D1S1	0.151	0.004	0.066319
D2S2	0.155	0.003	0.051057
D3S3	0.15	0.007	0.115290
D4S4	0.154	0.006	0.101455
D1Dr1	0.151	0.008	0.132638
D2Dr2	0.155	0.008	0.136152
D3Dr3	0.15	0.01	0.164700
D4Dr4	0.154	0.009	0.152183

Table 5.5 - Analysis of relative water content (RWC) in control and stressed *E.coracana* plants

Treatment	FW	TW	DW	RWC
Control1	0.049	0.0512	0.046	57.6923076
Control2	0.0487	0.0535	0.038	41.1764705
Control 3	0.053	0.058	0.048	71.4285
D1H1	0.0367	0.0458	0.0256	54.9504950
D2H2	0.032	0.0423	0.022	49.261083
D3H3	0.0357	0.049	0.025	44.58333
D4H4	0.02789	0.0367	0.0255	21.339285
D1S1	0.037	0.04	0.035	40
D2S2	0.047	0.0605	0.042	27.02702703
D3S3	0.063	0.078	0.059	21.05263158
D4S4	0.0491	0.0678	0.0473	8.780487805
D1Dr1	0.038	0.042	0.033	55.5555
D2Dr2	0.039	0.0456	0.0324	50
D3Dr3	0.026	0.0356	0.021	34.24657534
D4Dr4	0.029	0.0398	0.0236	33.33333



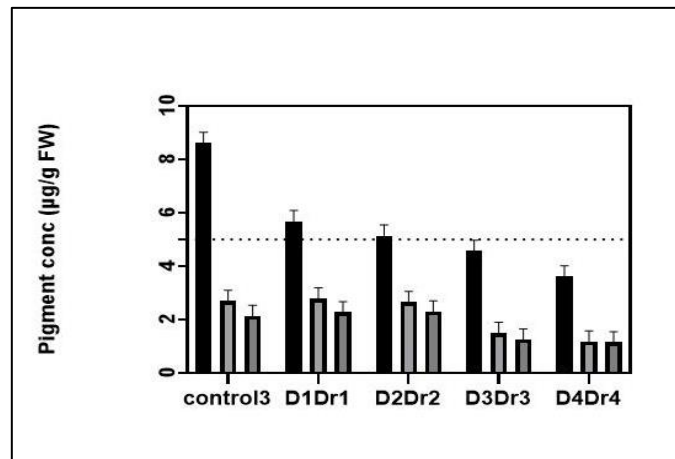
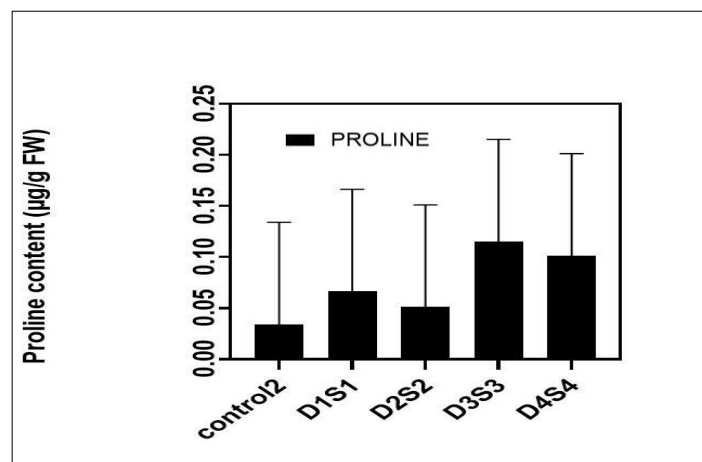
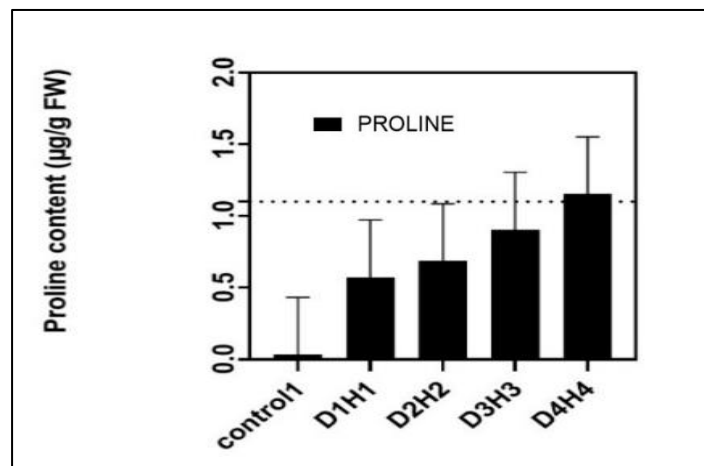


Fig 5.11 Graphical representation of pigment assay of stress treated plants with respect to control using Graph pad prism



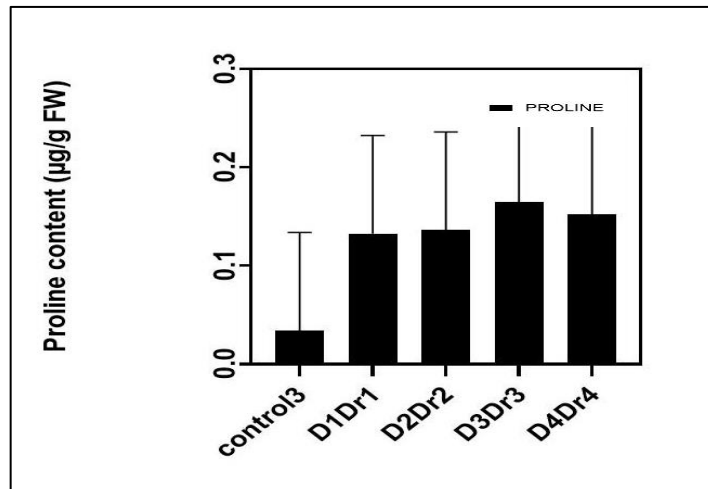
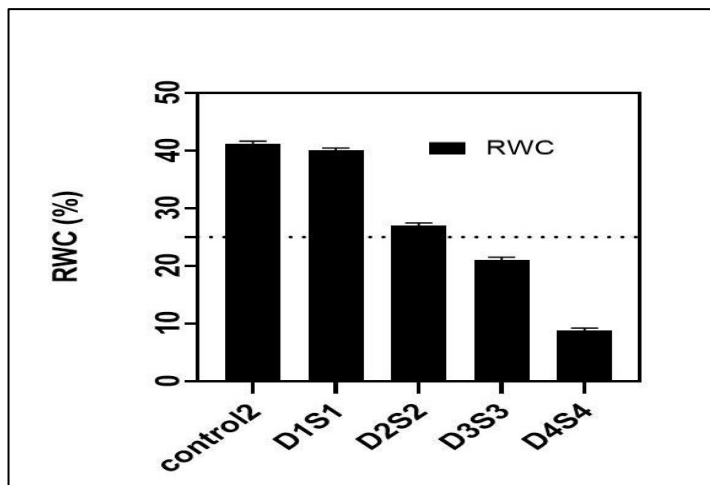
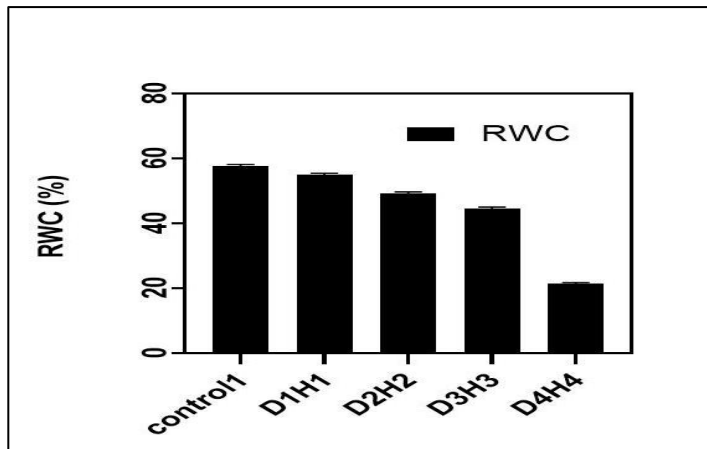


Fig 5.12 Graphical representation of proline assay of stress treated plants with respect to control using Graph pad prism



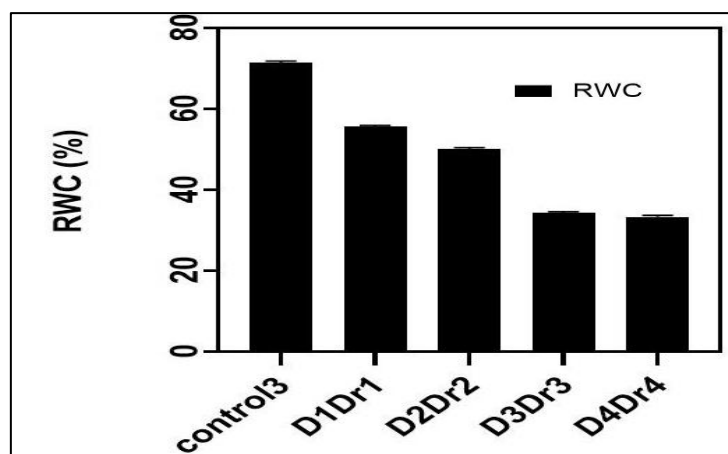


Fig 5.13 Graphical representation of RWC of stress treated plants with respect to control using Graph pad prism

5.9 STATISTICAL ANALYSIS:

paired t- test for pigment assay:

A) Between the control and heat treated samples- Chl A

Null Hypothesis (H0): There is no significant difference in chlorophyll A content between control and treatment plants.

Alternative Hypothesis (H1): There is a significant difference in chlorophyll A content between control and treatment plants.

Table 5.6 t-test for Chl A of heat treated plants

CONTROL	TREATED PLANTS	DIFFERENCE(d)	DIFFERENCE OF MEANS(d – d̄)	(DIFFERENCE OF MEAN)^2
8.0908	5.8765	-2.214	0.95825	0.8079

8.9975	5.58576	-3.411	-0.23875	0.057
8.6233	4.5546	-4.068	-0.89575	0.8023
6.7746	3.7779	-2.996	0.17625	0.03106

$$S.D = \sqrt{\frac{\sum(d-\bar{d})^2}{n-1}} = 0.752 \quad \text{mean of difference} = 3.17225$$

$$t \text{ calculated} = \frac{\bar{d}\sqrt{n}}{S.D} = -8.4368$$

Calculated t-value: -8.4368 , degrees of freedom =3

Critical t-value (for $\alpha = 0.05$, one-tailed test): 3.182

Therefore, we reject the null hypothesis and we accept the null hypothesis

B) For chl B-

Null Hypothesis (H0): There is no significant difference in chlorophyll B content between control and treatment plants.

Alternative Hypothesis (H1): There is a significant difference in chlorophyll B content between control and treatment plants.

Table 5.7- t-test for Chl B of heat-treated plants

CONTROL	TREATED PLANTS	DIFFERENCE(d)	DIFFERENCE OF MEANS(d - $\bar{d}$)	(DIFFERENCE OF MEAN)^2
2.865504	1.75702	-1.108	0.007	0.000049
2.9415	1.94829	-0.9933	0.1217	0.01481
2.7007	1.39223	-1.3084	-0.1934	0.0374
2.204	1.1527	-1.0513	0.0637	0.00405

S.D= 0.134

t calculated =-16.641

Critical t-value (for $\alpha = 0.05$, one-tailed test): 3.182

Therefore, we reject the null hypothesis and we accept the null hypothesis

C)For carotenoid-

Null Hypothesis (H₀): There is no significant difference in Carotenoid content between control and treatment plants.

Alternative Hypothesis (H₁): There is a significant difference in Carotenoid content between control and treatment plants.

Table 5.8- t-test for Carotenoid of heat-treated plants

CONTROL	TREATED PLANTS	DIFFERENCE(d)	DIFFERENCE OF MEANS(d – d̄)	(DIFFERENCE OF MEAN)^2
2.6295	1.6601	-0.9694	0.1695	0.028
2.33431	1.9349	-0.3994	0.4005	0.1604
2.1399	1.2037	-0.9362	-0.1363	0.0185
1.8772	0.9825	-0.8947	-0.0948	0.0088

S.D= 0.2304

t calculated = -6.943

Critical t-value (for $\alpha = 0.05$, one-tailed test): 3.182

Therefore, we reject the null hypothesis and we accept the null hypothesis

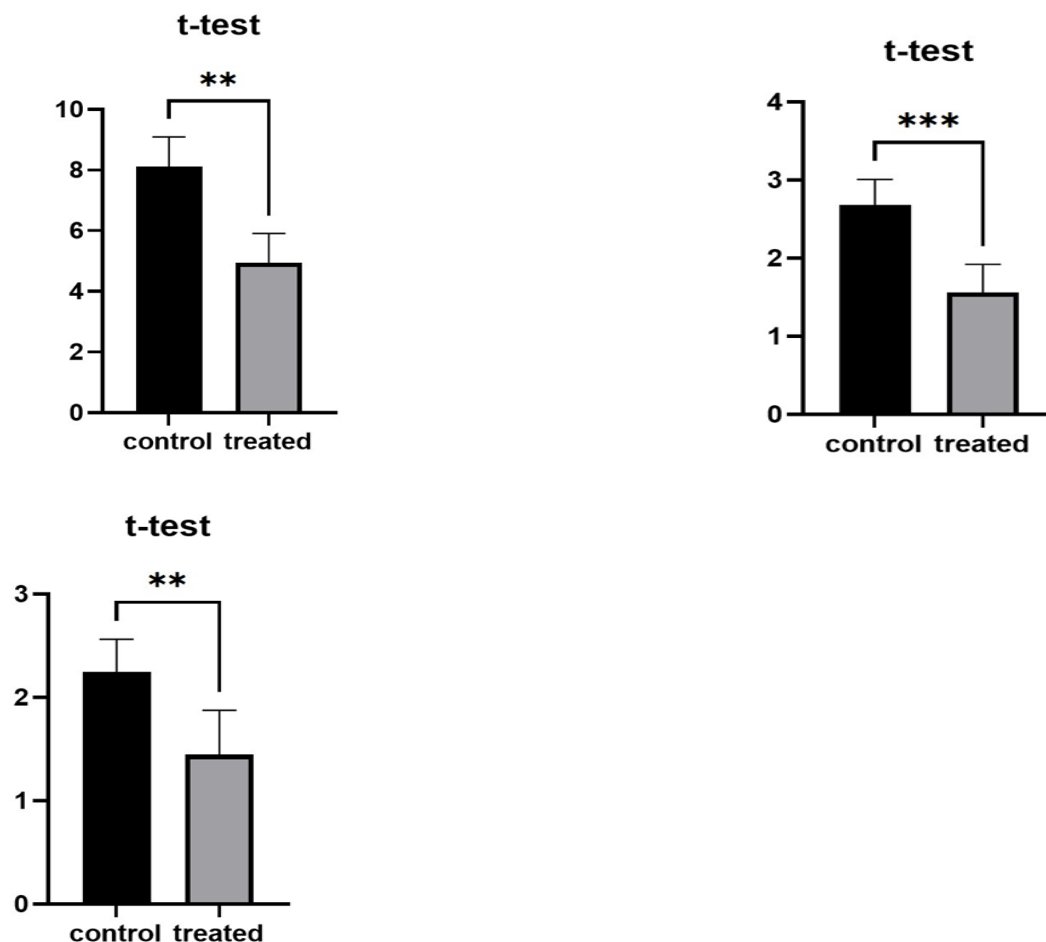


Fig 5.14-Graphical representation of t-test of heat-treated plants using Graph pad prism
Table 5.9- Correlative analysis

Variable 1	Variable 2	Correlation coefficient
Chlorophyll A	Chlorophyll B	0.976
Chlorophyll A	Carotenoid	-0.965
Chlorophyll A	Proline	-0.976
Chlorophyll A	FW	-0.203
Chlorophyll B	Carotenoid	-0.987
Chlorophyll B	Proline	-0.987
Chlorophyll B	FW	-0.126
Carotenoid	Proline	-0.945
Carotenoid	FW	-0.230
Proline	FW	0.060

FORMULA:

$$r = \frac{\sum(X-\bar{X})(Y-\bar{Y})}{\sqrt{\sum(X-\bar{X})^2} \sqrt{\sum(Y-\bar{Y})^2}}$$

Where, $\bar{X}$ = mean of X variable

$\bar{Y}$ = mean of Y variable

5.10 RT-PCR ANALYSIS OF EcJMJ GENES;

The RT-PCR results indicate distinct gene expression responses under different stress conditions. Under salinity stress, most JMJ genes were downregulated, including EcJMJ5, EcJMJ6, EcJMJ7, EcJMJ10, EcJMJ12, EcJMJ13, and EcJMJ15. Conversely, under drought and heat stress, these genes were predominantly upregulated. Specifically, EcJMJ5, EcJMJ6, EcJMJ7, EcJMJ10, EcJMJ12, EcJMJ13, and EcJMJ15 exhibited upregulation in response to both drought and heat stress conditions.

In birch, cold stress induces upregulation of eleven genes, while members of the JHDM3/KDM4 family are downregulated. Conversely, heat stress in maize leads to significant upregulation of seven ZmJMJ genes and downregulation of six others. Wheat shows increased expression of nine TrJMJ genes under drought stress, with specific genes responding rapidly. In *Oryza* species (Chowrasia et al., 2018), OsJMJ-C14 is upregulated under drought, while under salt stress, OsJMJ-C3 and OsJMJ-C13 are elevated in shoots. *Brassica napus* exhibits considerable upregulation of AtJMJ genes under drought, high-temperature, or salt stress. Mung bean displays varied expression patterns of VrJMJ genes in response to short-day therapy. Additionally, *Taraxacum koksaghyz* and tea plants exhibit altered expression of specific JMJ-C genes under stress conditions.

Litchi demonstrates differential expression of 17 HM genes during fruit abscission (Chowrasia et al., 2018). Moreover, sweet orange (Xu et al., 2015) and soybean display distinct

expression patterns of CsJMJs and GmJMJs, respectively, across various tissues and developmental stages. Furthermore, Citrus grandis and potato exhibit differential expression of specific JMJ-C genes under stress conditions. Arabidopsis and rice reveal active expression of 21 JMJ-C genes under typical developmental conditions. Additionally, *Gossypium hirsutum* and bamboo exhibit altered expression of GhJMJ-C and JMJ-C genes under osmotic and cold stress, and MeJA and ABA treatments, respectively. Notably, finger millet shows contrasting expression patterns of EcJMJ genes under salinity, drought, and heat stress, suggesting intricate regulatory mechanisms governing stress adaptation. These findings underscore the diverse roles of JMJ-C genes in stress responses and developmental processes across different plant species.

Table 5.10 -CDNA concentration using qubit fluorometer-

SAMPLE	CONCENTRATION (ng/microliter)
Control 1	3.84
Control 2	7.72
D1H1	25.6
D4H4	6.74
D1S1	2.76
D4S4	12.3
D1Dr1	1.62

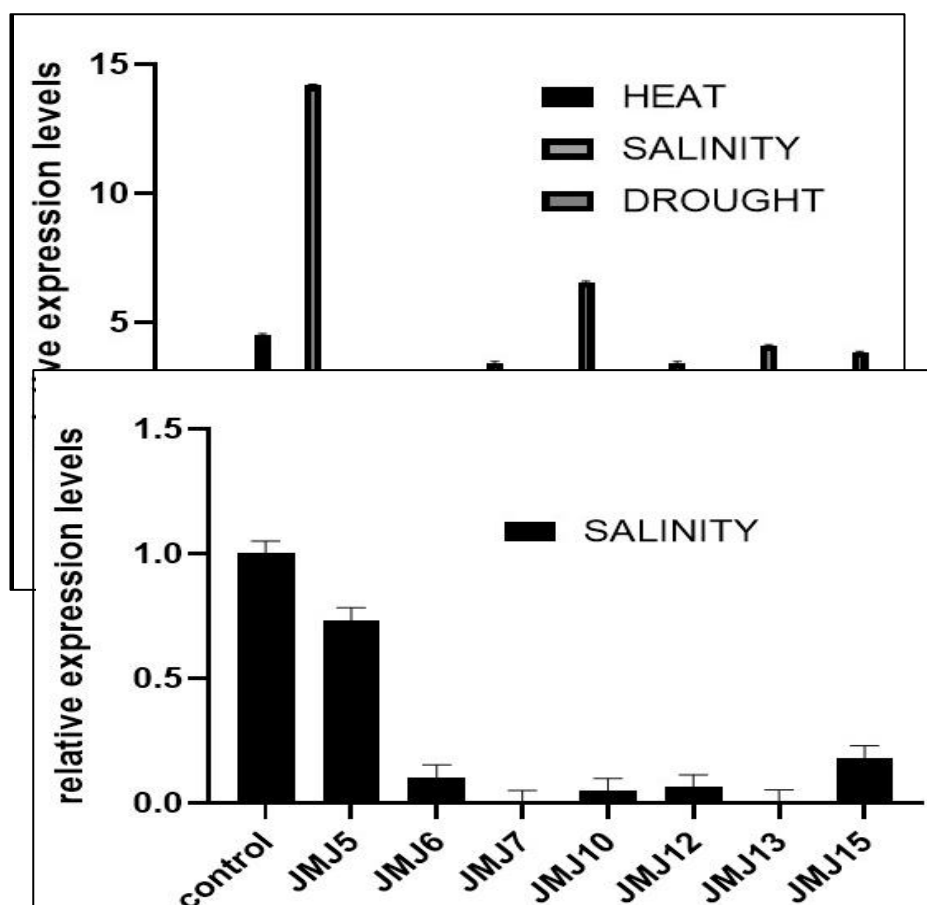
Table 5.11- primers used for RT-PCR

S no	genes	Left primer	Right primer	Tm(°C)	size
1	EcJMJ5	AGCAAAGGACTCCAAGTGCA	GGTCTCCTGCTTACGGACAG	58	119
2	EcJMJ6	CAGAGCAGCAGTTCCAGGAA	TGTTTTTGAGGCCCCACAGT	60	130
3	EcJMJ7	CGTCCTAGTGATCAGACGCC	CTGAGCCAGGCAGATTTGGA	60	103
4	EcJMJ10	TTGTGGCACGATGGAATCCA	GGCTCTGCTGTTGGTCGTAT	60	130

5	EcJMJ12	CACTACCGCGAGCATACCAT	TGCGATGCTGCTCTCTGTAG	59	129
6	EcJMJ13	ATGCAAATGATGTCCCGGGT	TCTCCCCATCATCCCCCATT	60	102
7	EcJMJ15	TGTTTTTGAGGCCCCACAGT	AGCAAAGGACTCCAAGTGCA	60	108
8	Ref – Actin	AACCCAAAAGCCAACAGGGA	CAGTTGTACGCCCACTAGCA	60	118

Table 5.12-expression analysis of genes

GENE	SALINITY	DROUGHT	HEAT
Control (Actin)	1	1	1
JMJ5	0.7334535502	14.18682785	4.518592038
JMJ6	0.1026350101	2.295805575	0.6056172941
JMJ7	0	3.426777378	0.06549297494
JMJ10	0.04830006166	6.547816098	0.4932758773
JMJ12	0.06257774611	3.437221449	0.247074948
JMJ13	0.002677902546	4.096239689	0.2290273782
JMJ15	0.1806603116	3.835573931	0.3163767108



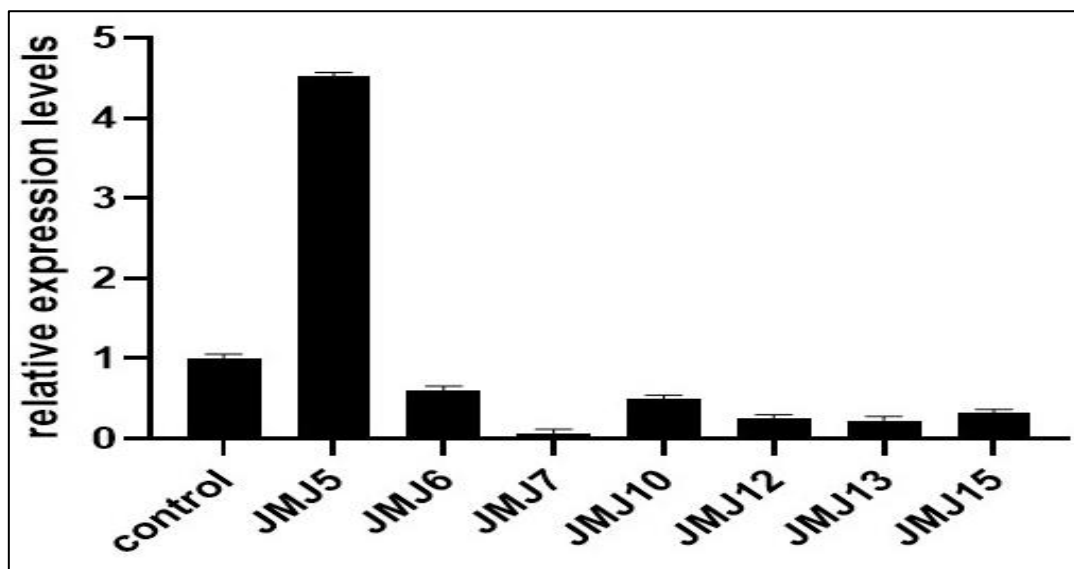
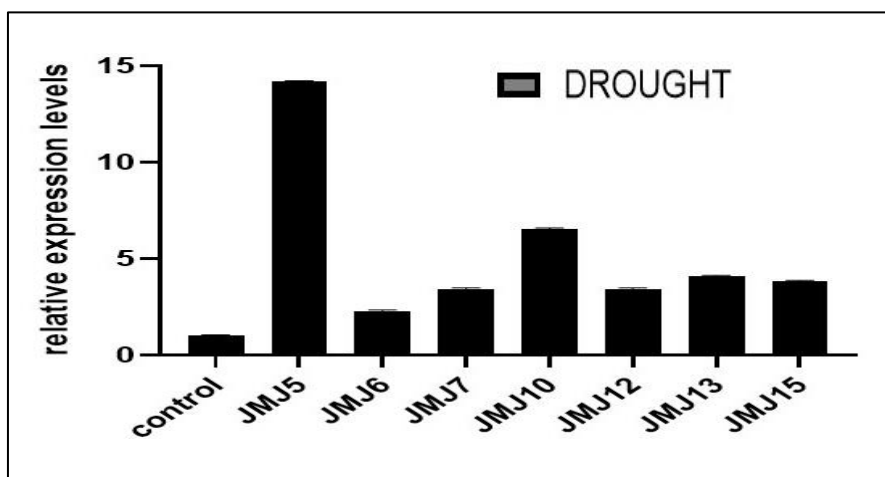


Fig 5.15-Graphical representations of expression a

CHAPTEF

SUMMARY AND

The genome-wide identification of JMJ-C genes in *E.coracana* revealed a total of 16 genes, designated as EcJMJ1 to EcJMJ16 based on homology with Arabidopsis genes. These genes exhibited variations in molecular weight, isoelectric point, and protein length. Subcellular

localization prediction indicated nuclear localization for many EcJMJ genes, potentially involved in histone demethylation to maintain genome integrity. Phylogenetic analysis grouped EcJMJ genes into different subfamilies, with varying member counts across subfamilies. Conserved motif and gene structure analysis highlighted distinct patterns among subfamilies, indicating sequence conservation and phylogenetic relationships. Chromosomal distribution showed even representation across nine chromosomes. Cis-element analysis of promoter regions revealed regulatory elements associated with phytohormones, light response, growth, development, and abiotic stress. The presence of hormone-responsive and stress-related elements suggests a role for EcJMJ genes in mediating plant responses to environmental cues. The comprehensive examination of plants subjected to heat, salinity, and drought stress revealed significant alterations in physiological and molecular responses. Stress conditions led to a notable decrease in chlorophyll A, chlorophyll B, and carotenoid concentrations, indicative of stress-induced damage to photosynthetic pigments. Conversely, there was a remarkable increase in proline content in stressed plants

compared to controls, suggesting its role as an energy source for stress adaptation. Weight analysis further supported stress-induced physiological changes, with a reduction in relative water content across all stress conditions, indicating compromised water retention abilities. The structural analysis of proteins plays a crucial role in predicting their interactions with other molecules or ligands and establishing sequence–structure–function relationships. The analysis of EcJMJ gene interactions unveils diverse functional associations across subfamilies. EcJMJ1-6 (KDM5) predominantly interact with ATP synthase and regulatory proteins like FIE and ARATH, likely influencing energy metabolism and developmental processes. EcJMJ9-11 (JARID1) exhibit interactions with histones and transcription factors, suggesting roles in chromatin regulation and gene expression. EcJMJ12-13 (KDM4) interact with proteins involved in plant defense, chromatin remodeling, and histone modification, indicating roles in stress responses and epigenetic regulation. These interactions underscore the importance of EcJMJ genes in fundamental cellular processes and provide insights into their potential roles in plant development and stress adaptation.

At the molecular level, RT-PCR analysis revealed distinct gene expression patterns among JMJ genes under different stress conditions. Salinity stress predominantly resulted in downregulation

of JMJ genes, while drought and heat stress led to their upregulation, particularly EcJMJ5, EcJMJ6, EcJMJ7, EcJMJ10, EcJMJ12, EcJMJ13, and EcJMJ15. From the present study, we conclude that EcJMJ15 is the candidate gene in *E.coracana* in response to abiotic stress as it had higher level of differential expressions. These differential expression patterns highlight the dynamic response of JMJ genes to environmental stressors, potentially influencing plant stress tolerance mechanisms.

Overall, the findings suggest that plants employ multifaceted strategies to cope with heat, salinity, and drought stress. Physiological adjustments such as alterations in pigment concentrations and proline accumulation are complemented by changes in gene expression, with JMJ genes playing a pivotal role in mediating stress responses. Understanding these molecular and physiological mechanisms provides insights into plant stress adaptation strategies and could inform future efforts aimed at improving crop resilience to environmental challenges

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Genome wide analysis of (jmc) gene family and their Expression Profile studies in response to abiotic stress

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Abstract

The JmjC gene family, which is distinguished by the conserved Jumonji C (JmjC) domain, plays a crucial role in the control of epigenetics and chromatin remodelling in plants due to their wide range of biological effects. This study delves deeply into the JmjC gene family's functional significance in altering epigenetic environments and directing chromatin dynamics. The family's diversity is shown by further classification into subgroups based on structural traits and evolutionary links, which may have ramifications for how the family adapts to stress. Abiotic stress, which includes issues like salt, dryness, and extremely high temperatures, poses serious risks to plant viability. This study emphasizes the necessity of understanding the molecular mechanisms controlling plant stress responses in order to develop appropriate crop enhancement strategies in light of the seriousness of these stressors. A comprehensive genome-wide investigation using bioinformatics techniques and phylogenetic studies aided to identify and classify JmjC genes in the plants. The dynamic expression patterns of JmjC genes in response to abiotic stress are revealed by subsequent high- throughput transcriptome analyses, which provide insights into their functional importance during stress as well as reveal prospective indicators for plant stress tolerance. We have also discussed the genomic context of JmjC genes, cis-acting elements in order to understand the transcriptional regulatory networks controlling the expression of these genes under abiotic stress. We now have a deeper understanding of the complex molecular dynamics of the JmjC gene family during stress reactions through this integrated strategy that combines genomes, transcriptomics, and regulatory genomics.

Keywords: *JmjC gene family, abiotic stress, transcriptomics*

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