CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

# Structural and Functional Analysis of Vascular Plant One-zinc Finger Proteins in *Zea mays*

by

Wania Idrees

A thesis submitted in partial fulfillment for the
degree of Master of Science

in the
Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2025

*I would like to dedicate my work to my beloved parents whose support and unconditional love have inspired me to reach this milestone and foundation of my every success.*

# CERTIFICATE OF APPROVAL

## Structural and Functional Analysis of Vascular Plant One-zinc Finger Proteins in *Zea mays*

by

Wania Idrees

(MBS241006)

## THESIS EXAMINING COMMITTEE

| S. No. | Examiner | Name | Organization |
|---|---|---|---|
| (a) | External Examiner | Dr. Joham Sarfraz Ali | NUMS, Rawalpindi |
| (b) | Internal Examiner | Dr. Sania Riaz | CUST, Islamabad |
| (c) | Supervisor | Dr. Sami Ullah Jan | CUST, Islamabad |

Dr. Sami Ullah Jan
Thesis Supervisor
September, 2025

Dr. Syeda Marriam Bakhtiar
Head
Dept. of Bioinfo. & Biosciences
September, 2025

Dr. Sahar Fazal
Dean
Faculty of Health & Life Sciences
September, 2025

# *Author's Declaration*

I, **Wania Idrees** hereby state that my MS thesis titled "**Structural and Functional Analysis of Vascular Plant One-zinc Finger Proteins in *Zea mays***" is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

**(Wania Idrees)**
Registration No: MBS241006

# *Plagiarism Undertaking*

I solemnly declare that research work presented in this thesis titled "**Structural and Functional Analysis of Vascular Plant One-zinc Finger Proteins in *Zea mays***" is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

**(Wania Idrees)**
Registration No: MBS241006

# *Acknowledgement*

Alhamdulillah! All praise and gratitude belong to Allah, the Lord of the worlds. I am deeply grateful to my supervisor, Dr. Sami Ullah Jan, for his invaluable guidance and constant support throughout my thesis journey. I also extend my heartfelt thanks to my family whose unwavering love and encouragement have been my strength. My sincere appreciation goes to my dear friend for her generous help and guidance, which have been instrumental in my progress. Additionally, I would like to express my gratitude to Dean Dr. Shear Fazal for his leadership and support, and to Dr. Mariam Bakhtiyar, Head of Department, for her continuous encouragement and insightful guidance. May Allah bless you all for your immense contribution to this journey.

**(Wania Idrees)**

# *Abstract*

The ever-increasing global population demands higher food production. However, achieving greater agricultural yields is hindered by various abiotic stresses. Biotechnological approaches employ genetic manipulation techniques to enhance crop plants' ability to withstand adverse climatic conditions. One promising target for such modifications is plant transcription factors (TFs), which are essential regulators of gene expression. Genetic manipulation of TFs can thus confer higher levels of stress tolerance. The *VASCULAR PLANT ONE-ZINC FINGER (VOZ)* family constitutes a unique class of zinc finger TFs known for their roles in flowering regulation, abiotic stress tolerance, and hormonal signaling. While *VOZ* genes have been extensively characterized in dicot species such as *Arabidopsis thaliana*, their presence and function in monocot crops like *Zea mays* (corn) remain largely unexplored. In this study, we conducted a genome-wide identification and comprehensive analysis of *VOZ* genes in *Zea mays*, using *Arabidopsis thaliana VOZ* (AtVOZ) genes as a reference. Our goal was to determine their putative role in abiotic stress tolerance and assess their suitability for inter- and intra-species genetic engineering. Two *VOZ* genes in *Zea mays*—*ZmVOZ1* and *ZmVOZ2*—were identified and further analyzed for their domain composition, physicochemical properties, gene structure, conserved motifs, promoter regions, phylogenetic relationships, and protein-protein interaction networks. Domain analysis confirmed the presence of the NAM superfamily, while physicochemical evaluations revealed that both proteins are acidic, hydrophilic, and nuclear-localized—traits typical of functional TFs. Comparative gene structure studies showed conservation with AtVOZ genes but also highlighted species-specific differences in exon-intron architecture and untranslated regions. Motif analysis identified ten conserved motifs across both *Zea mays* and *AtVOZ* proteins, suggesting evolutionary functional conservation. This study provides an in-depth structural and functional annotation of VOZ-TFs in *Zea mays*. The findings establish a strong foundation for future functional genomics research and have significant implications for improving stress resilience and developmental regulation in maize breeding programs by genetic engineering of VOZs as candidate genes.

**Keywords:** *VOZ*, *Zea mays*, abiotic stress, Transcription Factors (TFs), Functional annotation

# Contents

# List of Figures

# List of Tables

# Abbreviations

| | |
|---|---|
| **BLAST** | Basic Local Alignment search Tool |
| **GRAVY** | Grand Average of Hydropathicity |
| **GSDS** | Gene Structure Display Server |
| **ITOL** | Interactive Tree of Life Software v6 |
| **MEME** | Multiple Em foe Motif Elication |
| **NCBI** | National Centre for Biotechnology Information |
| **PlantTFBD** | Plant transcription Fcator Database |
| **VOZ** | Vascular Plant One-Zinc Finger |

# Chapter 1

# Introduction

## 1.1 Background

Zinc Finger proteins (ZFPs) form one of the most diverse and plentiful types of transcription factors of eukaryotic organisms. The proteins are identified by the zinc finger motifs present in them, in which a domain of the carvings is conserved, and maintained by one or more zinc ions, so that the proteins are able to associate with DNA, RNA, or proteins. Within the many groups of ZFPs, *Vascular Plant One-Zinc Finger (VOZ)* proteins constitute one plant-specific family that has become elevated as regulators of development, stress response, and signal transduction. *VOZ* proteins have, in recent years, been established as key regulatory components of the control of plant gene expression [1]. First characterised in the plant *Zea mays*, *VOZ* proteins have been linked to a number of different biological events such as flowering time regulation, abiotic and biotic stress tolerance, and phytohormone signalling. Even though the role of molecular functions and the structure of *VOZ* proteins in monocotyledonous crops, including *Zea mays* (maize), is acknowledged, it is still inadequately studied [2].

*Zea mays* is a crucial cereal crop worldwide as a major source of food, fodder and raw materials to industries. It is critical to learn about the regulatory networks of its growth and the genetic framework that comprises its response to stress so that crop output and fortitude can be boosted. As maize is an economically important crop, whereas *VOZ* proteins have a regulatory role, a structural and functional characterisation of *VOZ* genes in *Zea mays* is long overdue. This study aims to fill this gap by determining the structure (genomic and protein domain architecture) and, also, the function (expression patterns, physiological role) of the *VOZ* gene family in maize [3].

Among the characteristics of *VOZ* proteins, dual localisation in the nucleus and the cytoplasm is peculiar, which presupposes the dynamic regulation at various cellular locations. In Arabidopsis, *VOZ*1 and *VOZ*2 have redundant roles in flowering time regulation and are activated by environmental conditions, including temperature and photoperiod. These results indicate that *VOZ* proteins possibly coordinate developmental processes with environmental factors. But it is unknown whether the maize *VOZ* homologs have the same functions or have evolved differently in their regulative functions in the course of evolution in crop monocots. Analysis of their cellular localisation, expression patterns, and post-translational modifications in maize might provide valuable information about how transcriptional control evolved in higher plants [4].

The *VOZ* proteins also play roles in plant responses to abiotic stresses of plants, drought, salinity, and heat are the main limiting factors to maize productivity in the drought-prone climate variation regions. Investigations in other plants indicate that *VOZ genes* can regulate reactive oxygen species (ROS) properties, mediate stress-reliant gene mechanisms, and manage crucial phytohormones such as abscisic acid (ABA) and jasmonic acid (JA). Since the frequency of climate-induced stress cases is getting high, unravelling a possible role of *VOZ* genes in abiotic stress toleration in *Zea mays* can be of great interest regarding the production of climate-resistant maize varieties. Also, how the promoter elements and cis-regulatory motifs of *VOZ* genes relate to induction in response to special environmental conditions can be investigated [5].

Structurally, *VOZ* proteins tend to have only one zinc finger structure (though other highly conserved regions, such as the NAC (NAM, ATAF1/2, and CUC2) or bZIP-like domains that facilitate transcriptional activation, are also found). The structure and evolutionary preservation of these domains in *VOZ* proteins in maize are poorly documented in the literature. *Zea mays*-wide analyses and protein modelling might tell whether maize *VOZs* are structurally similar to those of other plant species, or whether it is the topology that was diversified during the lineage. This kind of structural characterisation, facilitated by a combination of sequence alignment, domain prediction, and phylogenetic profiling, would be needed to understand the invariant functional components and plan future studies in gene editing or functional disruption.

In addition, maize has a complicated and huge *Zea mays* with heavy duplications of genes, leading to the risk of functional redundancy or sub-functionalization between gene family members of *VOZ* and so on. Such genetic complexity requires a full analysis of the copies of the *VOZ* genes, their chromosomal position, exon-intron configuration and duplication to understand how the *VOZ* family has evolved in maize. Also, an in-depth analysis of the spatial and temporal expression of *VOZ* genes can be made on the basis of transcriptomic and proteomic data that can determine the stage-specific or tissue-specific functions during maize maturation, such as seed formation and root growth as well and leaf formation. The multi-dimensional insights will put together the study of *VOZ* transcription factors as the key players in maize biology [6]. This study is based on the principle of the main dogma of molecular biology and the gene-phenotype paradigm that genetic information, which is coded by DNA, is transcribed to RNA and finally translated into proteins, which subsequently regulate visible traits or phenotypes. Regulatory transcription factors like *Vascular Plant One-Zinc Finger* (*VOZ*) and proteins are of importance to regulate the expression of the genes, thus impacting all the developments, physiological reactions, as well as the adaptation of the plant to environmental stimuli. The theoretical hypothesis on which this study is based contains the assumption that *VOZ* genes in *Zea mays* behave as promoters of the organism's response by regulating the downstream gene networks and are associated with stress response, development, and metabolic cascades [15].

Identification and structural analysis of the genes constitute the initial element of this framework. The research will use state-of-the-art bioinformatics programs to target the identification of the *VOZ* gene family using the *Zea mays* . Conserved motifs and domains typical of the *VOZ* proteins (e.g. zinc finger region) will be identified with the use of tools (MEME, Pfam, InterPro). This is necessary to confirm the identity of *VOZ* genes, and to learn more about the molecular composition of the *VOZ* proteins, which impact their ability to bind DNA as well as functional specificity. Also, possible duplications of genes will be identified by chromosomal localisation, exon-intron regions, and exon structures should be used to determine the place of the *VOZ* genes in the *Zea mays* [16].

The second component of the framework is on phylogenetic and evolutionary analysis. This is being done so we can place the maize *VOZ* gene family in the larger evolutionary context by comparing its sequences with those similar. To be more exact, we compare the sequences *VOZ* gene family with those of other plant species that are related to the maize *VOZ* gene family, which are found in related plant species.

Various alignments of sequences will be performed through Clustal Omega or MUSCLE to determine conserved residues and patterns of evolution. The evolutionary relationships within *VOZ* genes across monocots and dicots will be inferred using phylogenetic trees constructed using such methods as the Maximum Likelihood or Neighbour-Joining algorithms. This comparative analysis will assist in answering the question of whether the *VOZ* genes in maize have either maintained ancestral functions or were diverted to have lineage-specific functions, and thus give an idea of their potential specialization and functional innovation.

The third key dimension is that of expression profiling, which can be done through the study of the expression of *VOZ* genes at the level of time and space in different tissues and in different environmental conditions. It will be achieved by mining publicly available RNA-seq data sets from repositories, e.g. NCBI Gene Expression Omnibus, GEO or MaizeGDB, and by interpreting quantitative real-time PCR (qRT-PCR) data when available. The analysis of expression profiles will be applied to determine how *VOZ* genes are regulated during such important developmental processes as seed germination or flowering, and in response to abiotic stresses such as drought, salinity, and high temperatures. The aim is to pinpoint the expression patterns that hint at functional roles of stress signalling pathways and developmental regulation, and help to target candidate genes based on them and conduct further experiments to validate or reject them.

The fourth and last part entails functional prediction, which makes use of computational tools and databases to determine the functions of *VOZ* proteins. The annotation of the *VOZ* genes will be classified into molecular function, biological process and cellular component categories using gene ontology (GO). The *VOZ* gene functionality will be established with reference to the involvement in metabolic pathways and signalling pathways using KEGG (Kyoto Encyclopedia of Genes and *Zea mays*s) pathway mapping. In addition, platforms such as the STRING or BioGRID will be used to construct protein-protein interaction networks to predict interaction partners and to ascribe the *VOZ* proteins to the larger context of cellular regulatory networks. These predictions will assist in clarifying the possible roles of *VOZ* genes in the process of stress acclimation, the regulation of hormones, and the transcriptome in maize [17].

Collectively, the systems-based approach combines structural characterization and evolutionary inference along with expression data and computationally predicted functions to give a tectonic view of the *VOZ* gene family in *Zea mays*. It facilitates the development of testable hypotheses concerning the role of *VOZ* genes in maize and a blueprint for further laboratory-based functional assays. Filling the gaps between genomics, bioinformatics, and systems biology, the framework enables an in-depth analysis, fundamental plant science, as well as targeted agricultural science.

## 1.2 Problem Statement

Improving plant's tolerance potential against abiotic stresses is crucial with reference to the increasing global population. The biotechnological approaches target those TFs and genes that are involved in abiotic stress tolerance mechanisms. One of such TFs is the *VOZ* that has been functionally characterized in several dicot and monocot species; however, its structure, diversity, evolutionary history, expression dynamics, and its suitability for abiotic stress tolerance remain largely unexplored in *Zea mays.*

## 1.3 Research Aim

The primary aim of this study is to conduct a comprehensive structural and functional analysis of the *VOZ* gene family in *Zea mays* to assess their potential as candidates for abiotic stress tolerance in *Zea mays.*

## 1.4 Research Objectives

To achieve the aim of the study, the following specific objectives have been formulated:

1. To identify *VOZ* gene family members in *Zea mays* genome.
2. To analyze structural and functional features of *ZmVOZs* and compare with *AtVOZ*s.
3. To explore the phylogenetic relationships and evolutionary divergence of *VOZ* genes among monocots and dicots.

## 1.5 Scope of Study

This study focuses on the comprehensive structural and functional characterization of the *VOZ* gene family specifically in *Zea mays.* The scope encompasses the identification of *VOZ* gene family members within the maize genome and comparative analysis with well-characterized *VOZ* genes in model plants such as *Zea mays* [1], [2]. It includes detailed examination of gene structure, conserved protein domains, chromosomal localization, and duplication events to understand evolutionary patterns and potential subfunctionalization in maize's complex genome [3], [16], [30].

Further, the study investigates expression patterns of *VOZ* genes in various tissues, developmental stages, and under abiotic stress conditions like drought and salinity through transcriptomic data mining and qRT-PCR validation where available [5], [25]. Functional prediction using bioinformatics tools like GO annotation and protein interaction networks forms part of the scope, linking *VOZ* gene activity to stress tolerance mechanisms and developmental regulation [17], [50].

However, this study is primarily bioinformatics- and systems biology-based, with experimental validation limited to data analysis from public databases and literature. Laboratory-based functional assays and field trials for stress tolerance enhancement remain outside the immediate scope but are proposed as future directions [15]. The scope is thus tailored to provide a foundational understanding to guide targeted experimental research on *VOZ* genes in maize.

## 1.6 Impact on Society

The findings of this study hold significant potential for agricultural and environmental sustainability, particularly in regions affected by climate change and abiotic stresses such as drought, salinity, and heat — major factors limiting maize productivity worldwide [5], [50]. By elucidating the structure, expression, and functional roles of *VOZ* transcription factors, this research contributes to the identification of candidate genes that can be targeted in breeding programs or genetic engineering to develop stress-resilient maize varieties [3], [17].

Enhanced stress tolerance in maize not only stabilizes crop yields but also ensures food security for growing populations dependent on maize as a staple food and industrial raw material [3]. Improved stress resilience can reduce reliance on irrigation and chemical inputs, leading to more sustainable farming practices with reduced environmental impact [5].

Furthermore, understanding the molecular basis of plant adaptation mechanisms promotes the advancement of precision agriculture and biotechnology, empowering farmers and policy-makers to implement scientifically informed strategies [15]. In the long term, this study supports efforts to mitigate the socioeconomic effects of climate variability on vulnerable farming communities by contributing to the development of maize cultivars with improved performance under stress [50].

# Chapter 2

# Literature Review

## 2.1 Introduction to *Zea mays* (Corn)

### 2.1.1 Historical Background and Domestication

*Zea mays.*, commonly known as maize or corn, is one of the earliest domesticated cereal crops, with its origin tracing back over 9,000 years to southern Mexico. Genetic and archaeological studies have confirmed that maize was domesticated from its wild ancestor teosinte, undergoing a series of genetic changes that resulted in the modern cultivated maize [1]. Through selective breeding, maize evolved from a small-seeded wild grass to a globally cultivated cereal crop with high-yielding potential and broad adaptability [2].

### 2.1.2 Taxonomy and Botanical Characteristics

Maize belongs to the family Poaceae, subfamily Panicoideae, and genus Zea. Its species, *Zea mays* ., is characterized by its tall annual growth, fibrous root system, cylindrical stem with distinct nodes, long linear leaves, and monoecious flowering system with tassels (male flowers) and ears (female flowers) [3]. The kernel is a caryopsis, typically yellow or white in color, and serves as a key source of carbohydrates and nutrients.

### 2.1.3 Life Cycle and Reproductive Biology

Maize is an annual, cross-pollinated crop with a life cycle that includes vegetative and reproductive phases. The reproductive stage involves tassel emergence, pollination (mainly wind-mediated), and grain formation.

The reproductive stage involves tassel emergence, pollination (mainly wind-mediated), and grain formation. The synchrony of silk emergence and pollen shedding is critical for successful fertilization.

Maize's reproductive flexibility and sensitivity to environmental cues have made it a model system for genetic and reproductive biology studies [4].

## 2.2 Global Importance of Corn

### 2.2.1 Role in Global Food and Feed Systems

Maize (*Zea mays*) stands as one of the most essential cereal crops worldwide, sharing prominence with wheat and rice. Its cultivation spans over 200 million hectares globally, contributing significantly to the global food supply [5].

As a staple food, it is consumed directly by humans in various forms, such as cornmeal, tortillas, grits, and popcorn, particularly in regions like Sub-Saharan Africa, Central America, and parts of Asia. More importantly, maize plays a central role in livestock production systems as a major source of feed for poultry, cattle, and swine due to its high-energy carbohydrate content and digestibility.

In many industrialized nations, over 60% of maize produced is utilized for animal feed, supporting large-scale meat and dairy production [16]. Additionally, maize-based feed products such as silage and high-moisture corn are critical for year-round livestock nutrition, reinforcing its contribution to both food security and economic sustainability.

FIGURE 2.1: Global corn production [16]

### 2.2.2 Economic Significance in the Agricultural Industry

Economically, maize is a cornerstone of global agriculture. It is a leading traded commodity in the international market and a crucial part of the agricultural GDP in countries such as the United States, China, Brazil, Argentina, and Ukraine [6]. The versatility of maize makes it highly valuable in various industrial sectors. For instance, maize is the primary feedstock in bioethanol production, particularly in the United States where approximately 40% of the corn yield is processed into renewable fuel, contributing to national energy strategies and carbon emission reduction [17]. Furthermore, maize derivatives are extensively used in the food processing, pharmaceutical, and textile industries. Products such as corn gluten, corn oil, modified starches, and fermentation-based chemicals are derived from maize and are used globally. The financial value of these by-products adds to the profitability of maize and influences national policies on agricultural subsidies, export-import regulations, and sustainable farming incentives [18]. Its global demand continues to grow in alignment with population increase, urbanization, and the shift toward processed and animal-based diets.

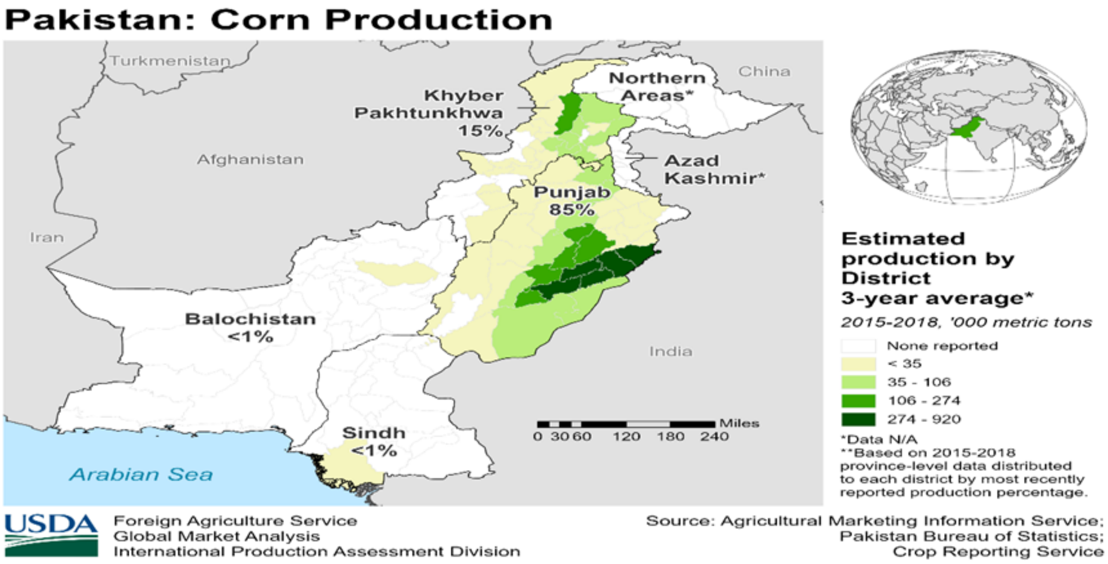
### 2.2.3 Nutritional and Industrial Applications

From a nutritional perspective, maize is a vital energy source, providing high amounts of carbohydrates, particularly starch, which accounts for 70–75% of its dry weight. It contains about 8–11% protein and small amounts of fats and essential micronutrients, including vitamin B-complex (thiamine, niacin, riboflavin), magnesium, and phosphorus [7]. While it lacks certain essential amino acids like lysine and tryptophan, biofortified maize varieties, such as Quality Protein Maize (QPM), have been developed to improve its nutritional profile [19]. Industrially, maize holds a prominent place in the production of diverse products. Corn starch is used as a thickening agent in the food industry, and high-fructose corn syrup is a common sweetener in beverages and processed foods. Corn oil is widely used in cooking and in the production of margarines and shortening. Moreover, maize is a raw material for the synthesis of biodegradable plastics, adhesives, and pharmaceuticals. Its wide-ranging industrial uses enhance its importance not only as a food crop but also as a key component in manufacturing and renewable energy sectors [20].

## 2.3 Corn Production in Pakistan

### 2.3.1 Overview of Corn Cultivation Areas in Pakistan

In Pakistan, corn (*Zea mays.*) holds the position of the third most important cereal crop after wheat and rice, both in terms of area under cultivation and its contribution to the national economy. It is widely cultivated across diverse agro-climatic zones, including the provinces of Punjab, Khyber Pakhtunkhwa (KP), Sindh, and Balochistan. Punjab has emerged as the leading corn-producing province, primarily due to the adoption of hybrid corn, improved agronomic practices, and mechanization. In contrast, KP continues to rely more on traditional farming systems and open-pollinated varieties. The rapid commercialization of corn cultivation in central and southern Punjab has been facilitated by access to certified hybrid seeds, better irrigation infrastructure, and linkages to agro-based industries [8]. These variations in regional practices highlight the dual nature of corn farming in Pakistan traditional and subsistence-based in some areas, while highly commercial in others.


Pakistan: Corn Production

Foreign Agriculture Service
Global Market Analysis
International Production Assessment Division

Source: Agricultural Marketing Information Service; Pakistan Bureau of Statistics; Crop Reporting Service

Figure 2.2: Corn production in Pakistan [8]

## 2.4 Trends in Yield and Production Statistics

Over the past decade, corn production in Pakistan has seen a remarkable upward trajectory. According to recent data from the Pakistan Bureau of Statistics, corn is cultivated on more than 1.7 million hectares nationwide, yielding over 10.6 million metric tons annually [9]. The national average yield has climbed to approximately 6.3 tons per hectare, positioning Pakistan among the top-performing corn producers in South Asia. Punjab, with over 80% share in national production, has achieved even higher productivity levels, largely due to widespread adoption of hybrid seeds, use of chemical fertilizers, and efficient crop management practices. The increasing demand for corn in the poultry feed, starch, and bioethanol industries continues to push both the acreage and yield of corn cultivation in the country [8].

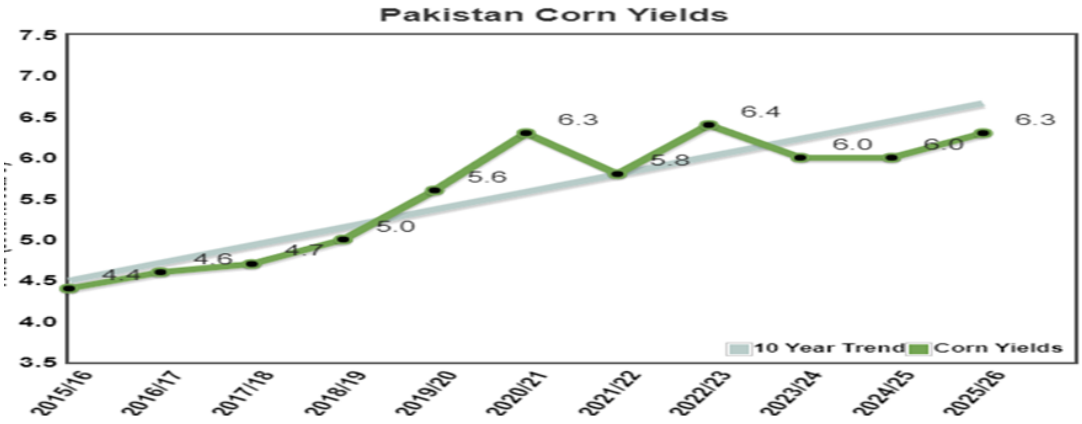


FIGURE 2.3: Pakistan corn yield in last 10 years [8]

### 2.4.1 Major Corn Varieties Grown in Pakistan

Pakistan grows a diverse range of corn varieties, which include both open-pollinated varieties (OPVs) and hybrids. In traditional farming areas such as KP and northern regions of Punjab, farmers continue to grow OPVs like Azam, Sarhad White, and Jalal due to their adaptability to local conditions and seed-saving potential [10]. On the other hand, commercial agriculture in central Punjab heavily relies on high-yielding hybrids developed by both multinational and local seed companies. Common hybrid varieties include Pioneer's P33B50 and P30F19, Syngenta's NK-8441 and NK-8711, and Monsanto's DK-6714 and DK-9108. These hybrids are engineered for traits such as early maturity, disease resistance, drought tolerance, and uniform cob development, making them suitable for mechanized harvesting and large-scale industrial use.

### 2.4.2 Challenges in Corn Farming: Biotic and Abiotic Stress

Despite the advancements in seed technology and agronomic management, corn farming in Pakistan still faces significant challenges. Abiotic stressors such as drought, heatwaves, erratic rainfall, and declining soil fertility are major constraints on productivity, especially in rainfed and resource-poor regions [11]. At the same time, biotic stressors including insect pests like the stem borer (*Chilo partellus*), fall armyworm (*Spodoptera frugiperda*), and aphids, as well as fungal diseases like Fusarium wilt and Ustilago maydis (common smut) cause considerable yield losses each year. Moreover, due to insufficient awareness and limited access to integrated pest management (IPM) practices, farmers often misuse agrochemicals, further compounding environmental and health issues. Climate change has exacerbated these stressors, necessitating the development of stress-resilient corn varieties and improved extension services for farmers.

### 2.4.3 Government Policies and Technological Interventions

The Government of Pakistan, in collaboration with international organizations such as CIMMYT and various private-sector stakeholders, has initiated several programs to promote sustainable corn production. These efforts include subsidizing certified hybrid seeds, improving access to quality fertilizers, and launching farmer education programs focused on crop management and post-harvest practices [12]. The Pakistan Agricultural Research Council (PARC) and provincial agricultural departments have also played a role in introducing climate-resilient and region-specific corn hybrids. Additionally, the adoption of technologies such as precision agriculture, drip irrigation, and mobile-based agricultural advisories is slowly gaining traction. These policies and technological interventions have collectively improved national corn productivity and are paving the way for a more resilient and profitable corn farming system in Pakistan

## 2.5 Corn as a Model for Functional Genomics

### 2.5.1 Significance of *Zea mays* in Genetic Studies

Corn (*Zea mays* L.) has been at the forefront of plant genetics and functional genomics for over a century. Its significance in scientific discovery began with the pioneering work of Barbara McClintock, who used corn cytogenetics to discover transposable elements, also known as “jumping genes.” Her groundbreaking studies on chromosome breakage and the movement of genetic elements revealed the dynamic nature of the genome and earned her a Nobel Prize in Physiology or Medicine in 1983. These discoveries not only revolutionized our understanding of gene regulation but also established corn as a powerful model organism in genetic research. Additionally, the high level of genetic variation found among corn inbred lines, landraces, and wild relatives makes it an ideal system for dissecting complex quantitative traits and understanding gene-by-environment interactions [13].

### 2.5.2 Availability of Genomic Resources and Databases

The widespread availability of high-quality genomic resources has solidified corn's position as a model species for functional genomics. The publication of the B73 reference genome marked a major milestone in corn research, providing a foundational sequence for gene identification, structural analysis, and functional annotation. Since then, pan-genomic studies have incorporated diverse germplasm to better capture the full range of genetic variability in the species. Multiple platforms now provide open-access genomic tools and datasets, such as MaizeGDB, which serves as the central repository for corn genetic and molecular data. Other major resources include Gramene and Ensembl Plants, which offer gene expression databases, functional annotations, comparative genomics tools, and synteny maps with other grasses. These platforms are essential for researchers performing in silico analysis, identifying gene families, and studying gene evolution and expression under different developmental and stress conditions [14].

### 2.5.3 Use of Corn in Gene Function Analysis and Crop Improvement

Corn continues to serve as a versatile system for gene function validation and molecular breeding. With the advent of next-generation sequencing and genome-editing technologies, researchers can now rapidly characterize gene function using tools such as RNA sequencing (RNA-seq), genome-wide association studies (GWAS), and CRISPR-Cas9-mediated gene editing. Mutant resources like the

UniformMu and EMS-mutagenized populations, as well as transgenic overexpression and RNA interference (RNAi) lines, have allowed researchers to link specific genes to phenotypic outcomes related to yield, abiotic stress tolerance, pathogen resistance, and nutrient use efficiency. These discoveries have not only deepened the biological understanding of corn physiology but have also accelerated the development of elite hybrids with desirable traits. Functional genomics in corn is thus directly contributing to global efforts to improve food security under climate-challenged agriculture [15].

## 2.6 Transcription Factors

Transcription factors (TFs) are master regulators in plants, coordinating complex developmental programs and responses to environmental stimuli [28]. Among these, zinc finger proteins (ZFPs) are notable for their highly conserved DNA-binding domains and regulatory flexibility [19]. A subfamily of ZFPs, known as *Vascular Plant One-Zinc Finger (VOZ)* proteins, has recently gained attention due to their unique structural configuration and multifaceted roles in plant growth, stress adaptation, and hormonal signalling [16].VOZ transcription factors have been primarily studied in dicotyledonous plants such as *Arabidopsis thaliana*, where they are known to regulate stress responses and developmental processes [17]. In Arabidopsis, *VOZ1* and *VOZ2* function as transcriptional regulators with nuclear localization properties under stress conditions [23].

Functional studies have demonstrated their role in controlling flowering time and drought tolerance [20]. Despite these advancements, limited research exists concerning the presence, diversity, and functions of *VOZ* genes in monocots, particularly in economically significant crops like *Zea mays* [29]. Maize is a staple cereal crop that contributes substantially to global food security and industry [5]. Its domestication from teosinte involved key genetic changes, particularly in genes such as Teosinte branched1, which altered plant architecture and productivity [1]. Genetic analyses have confirmed that maize underwent a single domestication event, supported by multilocus microsatellite genotyping [2]. The maize genome is characterized by extensive transposon activity, which influences gene expression patterns [3]. The role of transposons in gene silencing and activation has made maize a model for genome regulation studies [13].

Resources like MaizeGDB have facilitated functional and comparative genomic studies, enabling researchers to explore transcription factor families in depth [14].

Recent advancements in maize functional genomics have opened opportunities for characterizing novel gene families, including *VOZ* [15]. Studies in other cereals, such as rice and wheat, indicate *VOZ* genes are also involved in abiotic stress regulation [24].

In maize, transcriptome mining has identified several putative *VOZ* gene sequences with conserved domains, suggesting functional relevance [29]. These genes exhibit potential involvement in responses to abiotic stress and developmental signaling pathways [30]. Further research is required to confirm their exact roles through experimental validation.Efforts to improve drought tolerance and yield in maize through hybrid breeding strategies have emphasized the importance of regulatory genes, including transcription factors like *VOZ* [10]. Several hybrids have been evaluated across Pakistan's agro-ecological zones, indicating that genetic regulation plays a crucial role in adaptability [11].

This is further supported by national maize performance reports detailing genotype-specific resilience and productivity [8].Additionally, *VOZ* genes in other species, such as soybean, have been shown to modulate processes like nitrogen fixation, hinting at broader regulatory potential across plant systems [25].

Comparative evolutionary studies reveal that *VOZ* proteins share conserved domains but differ in sequence motifs between monocots and dicots, indicating lineage-specific adaptations [27].The integration of *VOZ* gene studies with modern genome editing tools such as CRISPR presents new avenues for improving maize traits under stress conditions [31]. Comprehensive functional annotation and mutant analysis of these genes could reveal targets for developing climate-resilient maize varieties [18].

## 2.7 Plant Transcription Factors

Higher plant Transcription factors. The transcription factors of the higher plant are an eclectic collection of regulatory proteins that are important in environmental responses, cellular differentiation, and development [20]. Predominantly, these proteins will be classified based on the existence of conserved DNA-binding domains that assure them the ability to regulate transcription due to their affinity to the promoter regions of the target genes. In plants, a broad range of TF families have been identified over the years, WRKY, bZIP, NAC, MYB, and zinc finger proteins, involved in TFs with pathways and regulatory mechanisms of their own [21][22].

TF regulation of gene expression is part and parcel of the plant response mechanism to abiotic and biotic stresses. As an example, stress signalling regulation by NAC transcription factors in the form of ANAC019 was observed to be modulated by the transcription factors and also provides structural domain specificity as a regulator of this process [23]. In the same breadth, WRKY TFs have also been associated with pathogen defence, oxidative stress and hormone cross-talk in a number of plant systems [24]. These understanding points to the importance of transcriptional regulators in modulating gene expression and phenotypic plasticity in unfavourable environmental variables.

TF families are especially multiplied in maize and other monocots as a result of duplication of their *Zea mays*, and their regulation is involved at the level of intricacy that can be seen as a result of the flexibility of the plant to various agro-climatic areas. Recent transcriptomic studies in a closely related cereal, wheat, indicated extensive divergence of stress-responsive TF expression, indicating functional partitioning has occurred between gene copies [25][26]. This puts into perspective the possible complexity and redundancy of the maize *VOZ* gene family and the significance of having a structural and functional annotation as the first step to downstream uses as part of crop improvement. When multiple rounds of *Zea mays* duplications have occurred, as in the case of *Zea mays*, the transcription factor families such as the *VOZ* family tend to be expanded. This genetic redundancy can be a cause of functional divergence of which is important to understand in the analysis of how maize responds to stress or during its development. Accordingly, structural and functional profiling of maize TFs, such as VOZ, is a crucial resource in crop enhancement plans aimed at abiotic tolerance.

## 2.8 The *VOZ* family and Zinc Finger Proteins

Zinc finger proteins form one of the largest known families of eukaryotic transcription factors, which share a common structure of Zn-binding motifs with each promoting interaction with nucleic acid or protein [27][28]. These motifs normally bind a zinc ion with conserved cysteine and histidine residues in a coordination which provides the structure and the functionality. ZFPs in plants play a variety of roles, including seed maturation, stress tolerance, and especially the development of meristems [29].

The *VOZ* subfamily constitutes a special kind of ZFPs that is specific to the vascular plants. Initially identified in *Arabidopsis thaliana*, and known also for plants, this is a seven-member gene family with one Zn finger domain showing nuclear-to-cytoplasm differentials in response to physical cues [30]. It was successfully identified and characterised the two *VOZ* genes *VOZ1* and *VOZ2*, which were reported to be expressed in the vascular tissues and played roles in various developmental activities [31]. Their localisation to both the nucleus and cytoplasmic stress granules was later confirmed and proposed to have roles in both post-transcriptional regulation and stress memory, especially in response to heat shock [32].

The *VOZ* proteins are structurally non-classical about the classical C2H2 type ZFP. Although they maintain the zinc finger DNA-binding domain, they also possess non-canonical domains involved in their interaction with other proteins and their participation in signalling cascades. *VOZ1* and *VOZ2* in Arabidopsis physically associate with the photoperiodic flowering regulator CONSTANS protein. This association facilitates floral induction. These interactions imply that *VOZ* proteins could act as transcriptional co-regulators or scaffolding molecules, which are then able to combine many signalling molecules [33].

In addition to flowering, *VOZ* proteins are involved in the signalling of hormones and seed germination. The negative control of the phytochrome B-dependent seed germination in Arabidopsis by *VOZ1* and *VOZ2*, which further characterises these proteins to regulate the light-dependent growth.

Remarkably, this overexpression led to higher biotic stress tolerance and lower abiotic stress resistance, which suggested an intricate trade-off in stress metabolic pathways [35]. This ambivalence of usage highlights the need for analyses of expressions situated in context to understand their exact functions as drivers of plant adaptation [34].

VOZ homologs have been found in monocots, including wheat and soybean, even though the vast majority of known functional data refer to dicot systems. *GmVOZ1G* in soybean is expressed differently in drought and salt stress and plays a role in the increased tolerance ability [36].

The observed factors will indicate the existence of a stress-responsive role that is conserved in different species, and therefore, maize *VOZ* proteins may also have a similar action. Yet, until more specific structural and functional analyses in *Zea mays* are performed, their exact contributions are hypothetical.

In *Arabidopsis thaliana*, where *VOZ1* and *VOZ2* were first characterised, it is the model plant. These genes are reported to regulate the time of flowering based on their fusion with CONSTANS (CO) and cellular stress response through protein trafficking between the cytoplasm and nuclei.

Unlike Arabidopsis, *Zea mays* is a monocot whose *Zea mays* and possible duplication events are highly complicated, and could contribute to a diversification of functions within *VOZ* homologs. The comparative sequence analysis of both Arabidopsis and *Zea mays* will thus help explain conserved and lineage roles of *VOZ* proteins.

Figure 2.4: Zinc Fingers [23]

## 2.9 Structure Features of *VOZ* Proteins

VOZ proteins are structurally characterised by a unique one-zinc finger motif that differentiates them from other ZFPs known to harbour several zinc finger domains. Its canonical or typical structure, *VOZ* proteins contain a Cys2His2-type zinc finger domain, usually around the C-terminal end of the protein, which allows the protein to bind DNA or bind to other regulatory proteins [37]. *VOZ* proteins, despite having only one zinc finger domain, are reported to possess great transcriptional regulatory potential since they can form multi-protein complexes and can interact with components of signal transduction pathways.

Bioinformatic analyses have shown that *VOZ* proteins are modular and have three regions (usually) an N-terminal region, usually the part involving protein-protein interaction (which can make them fall into C1 ubiquitin proteins); a central acidic region that may make them transcriptional activators or repressors and a C-terminal zinc finger structure [38][39]. Such modularity implies that the *VOZ* proteins may also act as scaffolds that form transcriptional machines besides DNA-binding effectors. Recent studies of mutational analysis and fusion constructs of *VOZ1* and *VOZ2* in Arabidopsis have shown that they both are localised to the nucleus in normal conditions but will redistribute to cytoplasmic stress granules during heat stress, indicating that their subcellular localisation is regulated on a post-translational basis in response to a stress condition [40].

Regarding domain conservation, the alignment of the *VOZ* sequence by sequence comparisons of protein sequences across a range of species, including soybean, wheat, rice and maize, has suggested that the zinc finger domain is highly conserved, whereas variation exists in N-terminal sequences [41][42][43].

Such variable regions could be responsible for species-specific roles or differences in interacting with transcribing co-factors. The conformation of protein *VOZ* enables interactions with the stress granule-bound proteins because it is shifted in response to heat exposure, further signalling a dynamic regulative strategy [44].

More so, phosphorylation sites, predicted in the *VOZ* proteins as well as nuclear localisation signals (NLS), reveal that the transcription factors are highly regulated, at the post-translational and intracellular trafficking level [45][46].

*VOZ* proteins seem to reconcile the gap between developmental and environmental response pathways in higher plants, not only due to possible relocalisation under the stress conditions but also due to the possibility to mediate the regulation of the flowering time. Their structurally permissible mobility and versatility as proteins have been made so by their set conserved core domain and the flip-flop areas, which provide plasticity.

Although there have been a lot of advancements in dicots, there is little information on how structurally diverse *VOZ* proteins in monocots are, e.g., *Zea mays*. It is also proposed that maize *VOZ* proteins could have similar domain structures to their closely related cereals, Triticum aestivum, which could have also evolved to have monocot adaptation [47] [48] [49][50].

An immediate requirement is a *Zea mays*-wide structural annotation of maize to identify both the conservation of motifs and domains, post-translational regulatory sequences and the evolutionary divergence of *VOZ* proteins.

## 2.10 Model Plant Functional Roles of *VOZ* Transcription Factors

With a functional aspect, *VOZ* transcription factors have been involved in many biological processes, including development and stress responses. *VOZ1* and *VOZ2* were also shown to redundantly control flowering time through interaction with the master switch of the photoperiodic flowering pathway, the CONSTANS (CO) protein in *Arabidopsis thaliana* [16]. The interaction triggers *VOZ* proteins to enhance the expression of FLOWERING LOCUS T (FT) that creates an acceleration in floral induction under long-day conditions [22][23]. This places *VOZ* proteins at the centre of being key integrators of environmental cues, especially of light and developmental changes [16][22][23].

Surprisingly, *VOZ* proteins are also connected to the adverse regulation of some growth processes. As mentioned *VOZ1* and *VOZ2* inhibit seed germination through phytochrome interaction, which is a photoreceptive device in the developmental decision-making process in response to light [37]. This observation favors the notion that *VOZ* factors may have context-dependent action, as flower induction and inhibition of premature germination under unfavorable conditions are observed. This regulatory duality exemplifies their flexible regulation of growth based upon environmental cues [37].

Additional support of *VOZ* functionality is garnered by overexpression and knockout. It was demonstrated that expression of *VOZ2* was increased in plants which increased bacterial resistance but also conferred drought and heat intolerance [17]. These findings suggest a trade-off mechanism such that *VOZ* activity in one pathway may repress responsiveness in the other one, thus influencing overall fitness in a complex environment. Such contrasting functions ensure that *VOZ* proteins are a prime target of genetic manipulation, depending upon particular crop-related needs like stress tolerance or maximizing yield [17].

Functional conservation of *VOZ* genes has also been identified in monocots and dicots, e.g., soybean (Glycine max). It was noted that Gm*VOZ1*G was upregulated in responses to drought and salt stress, and when overexpressed, provided better tolerance phenotypes [25]. The results indicate the possibility of *VOZ* proteins regulating some common stress-defense genes like dehydration-responsive element-binding (DREB) factors or heat shock proteins, or ABA signaling pathway components, but exact effectors are yet to be identified [25].

The other functional component entails hormonal signaling. It was provided evidence indicating that *VOZ* transcription factors were positive regulators of salt tolerance in Arabidopsis by regulating salt through ion transport and stress signaling pathways [50]. Since ion homeostasis is essential to salinity and drought tolerance, *VOZ* proteins could also have a role to play in plant resilience via regulation of ion transporter genes (or vacuolar H^+-ATPases) [46]. With experimental confirmation, such functional roles can be projected to monocots, such as maize [46][50].

Although most functional studies have been done in model dicots, it is necessary to study whether *VOZ* TFs of *Zea mays* have a similar or different role in regulating development and stress response, as it is agronomically important and unique in its physiology as well. Taken together, these observations show that *VOZ* proteins are multifunctional regulators that can adjust developmental processes or stress responses based on internal and external factors. The problem is to unravel how the division of function between paralogous *VOZs* in complex crop *Zea mays* can be worked out, and whether tissue-specific or inducible expression patterns play a role in functional specialization [16][25][50].

VOZ genes are somewhat well characterized in *Arabidopsis thaliana*, where functional knockouts and overexpression lines show specific phenotypes in flowering and immunity. *Zea mays* has experienced polyploidy, increasing the prospect of subfunctionalization between homologs of VOZ. Functional extrapolation from Arabidopsis can guide hypothesis formulation on gene functions in maize, but experimental evidence of direct orthologous functions is necessary considering evolutionary divergence [30][34].

## 2.11 *VOZ* Proteins in Stress Resistance and Hormonal Control

One of the most important research areas with *VOZ* pertains to abiotic stress tolerance. Drought, high salinity, and extreme temperatures are unfavorable conditions that plants face, activating transcriptional regulators mediating adaptation. *VOZ* proteins regulate stress-related gene expression, *VOZ* genes are upregulated under stress and their overexpression enhances osmotic and ionic stress tolerance [25][50].

The molecular mechanism of VOZ-mediated stress tolerance might involve modulating important stress response genes directly or via hormonal pathways. *VOZ* genes have been linked to regulation of the abscisic acid (ABA) signaling pathway, essential in drought and salinity stress responses. *VOZ* proteins also influence cytokinin and ethylene signaling, regulating stomatal closure, antioxidant defenses, and osmoprotectant synthesis critical for survival under water shortage [50].

VOZ genes influence transcription cascades; overexpression links to activation of WRKY, NAC, and DREB transcription factors known in abiotic stress tolerance. Transcriptomic analyses in Triticum aestivum reveal *VOZ* gene upregulation under heat/drought and co-expression with redox enzymes and heat shock proteins [34].

Besides transcriptional control, *VOZ* proteins may regulate ion homeostasis and oxidative stress. They interact with proton-pumping pyrophosphatases like AVP1, maintaining vacuolar pH and ion homeostasis under salt stress [46]. This suggests *VOZ* proteins also act as non-transcriptional regulators of intracellular homeostasis. Their multifunctionality underlines importance in breeding for climate-resilient crops [46][50].

Challenges arise due to VOZ's dual role: overexpression can improve biotic stress tolerance but reduce abiotic stress tolerance. Expression level and condition-dependent studies are needed. Spatial and temporal expression analysis in *Zea* *mays* should be complemented by qRT-PCR and stress-inducible promoters for further detail [25][34][50].

## 2.12 Expression Patterns of VOZ: Spatial, Temporal and Stress-induced

The biological roles of *VOZ* genes remain incompletely understood; knowledge of their spatial, temporal, and stress-induced expression is crucial. *VOZ1* and *VOZ2* are expressed in various *Arabidopsis thaliana* tissues (leaves, flowers, seeds) and are dynamically regulated by abiotic and biotic stress, suggesting coordination of growth with environmental cues [16].

Studies indicate *VOZ* expression is stress-regulated. It was found Gm*VOZ1*G highly upregulated in soybean roots and leaves during salt and drought stress, implying a role in primary stress perception and signal transduction. Transcriptomic data validated by qRT-PCR supports *VOZ* involvement in abiotic stress mitigation [25].

In wheat, *VOZ* homologs show spatial and temporal expression differences during seed and flag leaf maturation. MicroRNA profiling suggests *VOZ* targets regulate seed filling and maturation. Temporal transcriptome profiling revealed homeologous *VOZ* genes exhibit distinct expression partitioning under heat and drought stress, indicating functional diversification [34].

Environmental regulation of *VOZ* gene expression in cereals like rice involves stress-responsive cis-regulatory elements (CREs), such as ABRE, DRE, and HSE, activating expression under drought, salinity, and heat stress. Maize *VOZ* promoters likely contain similar motifs, but functional verification is incomplete [24]. Platforms like Expression Atlas and MaizeGDB facilitate monitoring tissue-specific and stress-related gene expression [14][47]. However, maize *VOZ* gene expression mapping is incomplete—a significant gap since maize faces extreme field conditions. Using existing maize data can help define *VOZ* expression timing and tissue specificity, critical for functional understanding and crop improvement.

Co-expression analysis tools can reveal transcriptional networks and regulatory modules, highlighting VOZ's interaction with other transcription factors like WRKY, NAC, and bZIP [44].

## 2.13 Evolution and Phylogenetics of *VOZ* Genes

Understanding the evolution of *VOZ* transcription factors is key to grasping function conservation and divergence in plants. *VOZ* genes are specific to vascular plants, likely arising after divergence from non-vascular lineages. Their conserved zinc finger domain indicates common ancestry, while variable regions suggest species-specific functional diversification [34].

Phylogenetic studies show *VOZ* genes form distinct monocot and dicot clades with considerable homology within groups. *VOZ1* and *VOZ2* likely originated from a duplication event predating monocot-dicot divergence, with paralog retention suggesting subfunctionalization or neofunctionalization [22][30].

Wheat, closely related to maize, exhibits multiple homeologous VOZ-like genes with divergent stress expression, illustrating gene duplication and functional diversification. Maize, with its complex genome and ancient duplications, likely shows similar paralog diversification [34].Bioinformatics resources like PlantTFDB and NCBI CDD enable evolutionary analysis of transcription factors. PlantTFDB 4.0 for exploring motifs, phylogenies, and networks. Maize *VOZ* genes can be compared with orthologs to infer evolutionary rates, selection, and function shifts [30].

Intron presence and alternative splicing add evolutionary and functional complexity. Mattick emphasized introns' roles in transcript diversity and regulation. Maize *VOZ* genes might produce multiple isoforms with tissue- or stress-specific functions, a promising area for future research [40].Combined phylogenetic and evolutionary studies clarify *VOZ* structure-function relations across plants.

Maize *VOZ* genes cluster differently than *Arabidopsis*, reflecting distinct evolution since monocot-dicot split. Maize's multiple paralogs contrast with *Arabidopsis*'s

*VOZ1*/*VOZ2* pair. Domain conservation and expression comparison with *Arabidopsis* are necessary to determine function conservation or novelty [30][34].

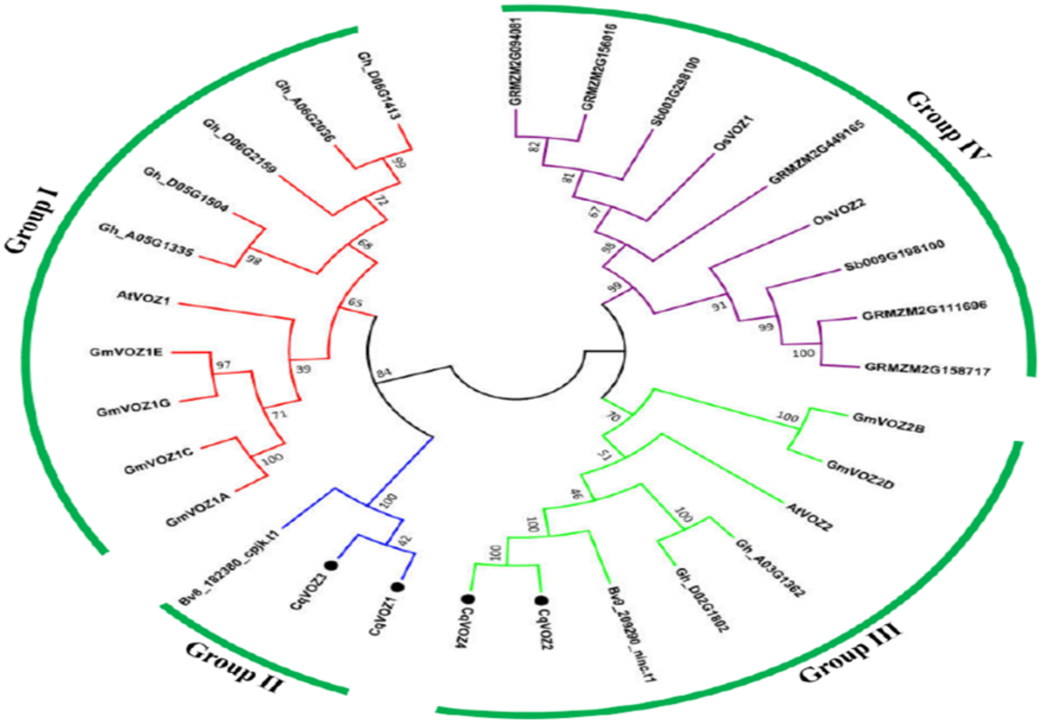


Figure 2.5: VOZ Protein in different plant species [2]

## 2.14 Monocots and *Zea mays* relevance of *VOZ* Proteins

Although intensive functional analysis of *VOZ* proteins has been done in model species of dicots, including *Arabidopsis thaliana*, little is known about their properties in monocots. This forms a major research challenge given the fact that monocot crops, such as maize, wheat, and rice, are the major staples across the world, and many of these crops are often subjected to environmental stress. The available information on wheat and soybean *VOZ* homologs forms a basis on the assumption of their possible role in maize, in addition to the understanding that it needs species-specific confirmation.

*Zea mays* has a large as well as complex level of *Zea mays* possess possessing a high level of repetitive elements as well as gene duplication to levels of the regulatory redundancy and plasticity. Thus, there is a possibility that multiple copies of *VOZ* have overlapping or differentiated functions within a single plant (maize).

Nevertheless, there has been no published study that has researched the full *Zea mays*-level, which has been able to provide the number of *VOZ* genes, their chromosomal location, as well as their structural diversity in maize, thus far. Such a dearth of simple annotation restricts the functioning of studies and makes the application of the *VOZ* proteins uneasily repaid in maize genetic engineering or breeding programs.

Since maize is central to the world agricultural practice and is also prone to climate change effects, the functional analysis of regulatory genes such as *VOZ* will be useful not only as a knowledge but also as an agricultural necessity. The *VOZ* proteins are particularly enticing targets due to multiple roles of *VOZ* proteins that include development and stress adaptation, and thus, potential candidates for manipulation to increase crop tolerance without reducing yield.

As *Zea mays* editing tools like CRISPR/Cas9 emerge and become available, *VOZ* genes might be identified, functionally confirmed as candidate *VOZ* genes, but edited to improve some trait characteristic like the time of flowering or drought resistance.

Availability of databases containing expression data, e.g. MaizeGDB and Expression Atlas, as well as high-throughput transcriptomic tools, can provide a mechanism to get started on mapping *VOZ* expression in maize In addition, networks comprising *VOZ* and other major regulatory proteins like CO, ABI5 or phytochrome B could be revealed by protein-protein interaction research- protein-protein interactions that are already well understood in Arabidopsis .

Therefore, conclusively, *VOZ* proteins are not very well exploited in the field of maize biology, with little research taking place. The cellular, structural, and phylogenetic analysis of their positions will be conducted to understand their specificities, structure, and dynamics in the evidence of their expression, which will be used to formulate their functional validation and improvement strategies in *Zea mays* crops.

## 2.15 VOZ Bioinformatics and Gene Expression Platforms

The era of high-throughput sequencing technologies and bioinformatics has completely transformed the field of plant molecular biology and enabled researchers to explore transcription factor families, such as VOZ, on a *Zea mays*-wide, system scale. These are computational platforms used to discover genes, identify motifs, predict domains, analyse evolution, and do expression profiling, and these are all underpinning studies in structure and function.

One of the key resources to perform transcription factor data mining is in PlantTFDB (Plant Transcription Factor Database) that provides integrated TF sequences of various plant *Zea mays*s, predicts domain structures, and allows species comparisons. PlantTFDB 4.0 as a complex resource uniting more than 80 plant *Zea mays* and including tools allowing the classification of *VOZ* proteins according to the prediction of Pfam and InterPro domains. On the *Zea mays* analysis, *VOZ* can be used by researchers to input candidate sequences to verify the identity of the domain and identify regulatory motifs, helping to energise the functionality, but before wet-lab confirmation.

Another very popular method of making predictions about *VOZ* protein functions is the use of gene ontology (GO) and KEGG pathway enrichment analysis of known annotations of homologous genes. The following GO categories were assigned to *VOZ* proteins, such as DNA-binding, response to abiotic stimulus and transcriptional regulation. Their participation in signal transduction and stress adaptation was also proposed by KEGG mapping. The identically used predictive methods might be applied in maize to suggest functional possibilities of uncharacterized *VOZ* proteins .

Co-expression network analysis is another useful method in *VOZ* research, where inferences of functional relationships between the variables are made based on common experiences of transcriptional amenities. The co-expression software allows the packaging of gene expression patterns under different conditions to allow clustering. Sent to transcriptomic data such as in Expression Atlas or MaizeGDB, this approach will be useful in identifying *VOZ* gene partners implicated in certain stress or development pathways This form of analysis is especially applicable in crops whose gene families are large, where redundancy makes it difficult to infer the usefulness based on their sequence alone.

RNA-seq data has taken the standard in carrying out gene expression profiling. Portals such as the Expression Atlas summarise normalisation gene expression measures across multiple studies and enable researchers to evaluate *VOZ* expression among non-paired tissues, developmental stages or drug treatment conditions [47]. The protocol by Livak and Schmittgen (2∧(-DELTA-DELTA)-CT method) coupled with qRT-PCR validation is a classic procedure of relative expression measurement involved in the analysis of *VOZ* genes, and it guarantees successful induction of genes by drought, salt or by any chemotherapeutic agent.

Regarding subcellular localisation and protein interactions, subcellular localisation can be predicted using e.g. WoLF PSORT, and protein interaction using e.g. STRING, to describe where *VOZ* proteins perform their work in the cell and which other proteins they are likely to interact with [29]. As an illustration *Arabidopsis VOZ2* relocated to stress granules when subjected to heat stress, which shows that this gene is involved in post-transcriptional regulation. These possibilities of computational modelling of these behaviours will enable researchers to derive some hypotheses that can be experimentally verified using confocal microscopy or yeast two-hybrid assay [29].

Announcement Resource like Maize DB and Ensemble Plants, which include vast genomic and transcriptomic information of *Zea mays*, allows researchers to carry out *VOZ* gene identification, motif prediction, and expression profiling on maize-specific data, and this now opens the scope of species-specific annotation and functional investigations. Finally, a combination of genomic and transcriptomic technologies with CRISPR/Cas9 gene editing systems creates additional possibilities in the validation of *VOZ* functionality. As soon as candidate *VOZ* genes are selected and linked to particular phenotypes or responses to stress, the specific gene editing can be utilised in order to validate the maize functionality. This has already demonstrated the potential in altering stress-related TFs in other crops [50].

## 2.16 Gap in Knowledge

Most of the work in describing *VOZ* transcription factors in dicots has grown at an immense rate; however, our knowledge with regard to the involvement of *VOZ* transcription factors in monocots (*Zea mays* included) is bleak. First, an exhaustive list of *VOZ* genes in maize has not been drawn, and their description in terms of duplication, position in the *Zea mays*, as well as structure, has not been studied in the *Zea mays*-wide context. Since maize has experienced a handful of genomic duplications, there is a great likelihood that several *VOZ* paralogs will be found, each specialised in a variety of functions. It is an urgent need to have a thorough in silico identification that involves annotation and prediction domains of the *Zea mays*.

Second, maize *VOZ* proteins are still hypothetical. Although Arabidopsis, soybean, and wheat data have implicated miRNA in flower regulation, stress response and hormone signalling [30] [32] [43], experimental verification of these claims in maize has been lacking. The challenge of functional redundancy among TFs in maize is also a factor that does not allow for obtaining a visible phenotype with one-gene knockout. Multiplex gene editing and overexpression techniques through CRISPR/Cas9 should be studied in the future to evaluate the effect of *VOZ* gene manipulation on growth and its ability to withstand stress.

Third, even though other related species have been explored concerning *VOZ* gene expression, little is understood concerning the spatial and temporal expression of maize. There is a lot of available expression data that has been established based on general stress transcriptomics and not necessarily pinpointed on *VOZ* genes. This complicates the determination of their contribution to developmental transitions, tissue-specific signalling or stress responses. To identify the expression in controlled stresses and during development, it is requisite to delineate specific expression analysis through qRT-PCR and promoter: GUS construct fusions.

The fourth gap on a large scale is the knowledge of the protein-protein interactions with *VOZ* proteins in maize. *VOZ* proteins are the interactors of CO and phytochrome B in Arabidopsis in a way that regulates the process of flowering and germination [30] [37]. Nonetheless, these interactions in maize are not characterised. Screening of maize-specific *VOZ* interactions by co-immunoprecipitation or yeast two-hybrid may reveal maize-specific interactors, including bZIP, NAC or WRKY proteins acting in maize signalling under stress [20] [23] [48].

Finally, *VOZ* proteins translated into practice in breeding are poorly studied. The application of *VOZ* genes in crop enhancement is difficult despite the capacities of the genes to offer multi-stress tolerance because the genes, when utilised in crops, will stimulate their biotic stress resistance and reduce their tolerance to abiotic stresses [43]. The regulatory pathways need to be dissected meticulously in the future to find context-specific promoters or variant genes that promote desirable traits that are not at the expense of yield or stress tolerance.

## 2.17 Summary

In this chapter, current literature about *VOZ* transcription factors was reviewed in light of structure, functionality, and plant regulation, where special attention was paid to *Zea mays*. Proteins that contain *VOZ* are distinct plant-type transcription factors with a single zinc finger and a multi-sorter regulatory potential. They are involved in the regulation of the flowering time, stress resistance, and hormone signalling. Arabidopsis functional studies, together with wheat and soybean, indicate that *VOZ* proteins are strongly controlled at transcriptional and post-transcriptional stages, their expression is induced in response to stress, and they are members of intricate signalling networks that link other transcription factors and phytohormones.

In spite of this progress, key gaps in our knowledge of *VOZ* proteins in maize exist. We know very little about their gene organisation, expression, evolutionary divergence and physiological implications. PlantTFDB, Expression Atlas, KEGG, etc, provide a reference to predict the structural as well as functional attributes of these elements; however, the true tests can only be done through experimentation. Incorporation of bioinformatics, gene expression profiling, phylogenetics, and CRISPR/Cas9-based approach in genetic modification of genes has potential in enabling maximum realisation of *VOZ* genes in maize to make maize more resilient to stress and productive.

The bridging of these gaps will play an important role in any crop improvement programs and broadening of the molecular toolbox to generate climate-smart solutions in maize and monocot crops in generation.

# Chapter 3

# Methodology

The methodological basis of the study is carried out on the basis of *in silico* methods aimed at targeting, annotating, and characterising the *VOZ* gene family in *Zea mays*. The *VOZ* transcription factors, well-characterised in the abiotic stress response and flowering in both *Arabidopsis thaliana* and *Oryza sativa* models, have not yet been explored fully in corn [4], [15], [30].

In the present study, a bioinformatics-guided approach is applied to the systematic analysis of *VOZ* genes in the corn *Zea mays*, including comparative genomics, sequence alignment, motif analysis, expression profiling and functional prediction applications.

In the present study, a bioinformatics-guided approach is applied to the systematic analysis of *VOZ* genes in the corn *Zea mays*, including comparative genomics, sequence alignment, motif analysis, expression profiling and functional prediction applications. Taking into account the size and the complexity of the maize *Zea mays*, which is characterised by several duplication events, a *Zea mays*-wide and comparative study is an absolute necessity [16], [25].

The methods used to achieve this end are as follows, and below each methodological step, an explanation is provided to develop an overall picture of the structure, evolution, and functionality of the (*VOZ*) genes in corn.

## 3.1 *Zea Mays*-Wide Identification of *VOZ* Genes in *Zea mays*

To identify the full complement of *VOZ* (*Vascular plant One-Zinc finger*) gene family members in *Zea mays*, the maize *Zea Mays* was accessed via NCBI. Reference sequences of known *VOZ* genes from *Arabidopsis thaliana* (*AtVOZ*) were used as queries in BLASTP and TBLASTN searches to screen the *Zea mays zea Mays* for putative *ZmVOZ* homologs.

Additionally, known *VOZ* domain profiles were retrieved from the Pfam database and used in HMMER-based searches to ensure domain-level validation of candidate proteins.

## 3.2 Retrieval of *ZmVOZ* Protein Sequences

Protein sequences of putative *ZmVOZ* family members were extracted from NCBI (https://www.ncbi.nlm.nih.gov), Phytozome (phytozome-next.jgi.doe.gov), and the Plant Transcription Factor Database (PlantTFDB: http://planttfdb.gao-lab.org/). The sequences were retrieved in FASTA format and archived for downstream computational and comparative analyses. *Arabidopsis VOZ* sequences were used as queries to confirm homology, and accessions were cross-referenced to avoid omission of gene isoforms or alternate transcripts.

## 3.3 Removal of Redundant *ZmVOZ* Sequences

To ensure non-redundancy and accuracy of the dataset, all retrieved *ZmVOZ* sequences were aligned using Clustal Omega. Sequences that were either duplicate entries across databases or identical due to transcript variants were filtered out. Only full-length, non-redundant protein-coding sequences were retained. Accession numbers were re-verified, and unique gene IDs were assigned to each *ZmVOZ* gene for consistency in further analysis.

## 3.4 Assigning Lab IDs and Sequence Similarity Analysis of *ZmVOZ* Genes

Each unique *ZmVOZ* gene was labeled with a specific lab identifier (Lab-*ZmVOZ1*, Lab-*ZmVOZ2*, etc.). A sequence identity matrix was generated using BioEdit and ClustalW to examine pairwise sequence similarity. Only sequences with ≥80% similarity across conserved domains were grouped for functional comparison. Divergent sequences below the threshold were excluded to maintain dataset specificity.

## 3.5 Physicochemical Characterization and Conserved Domain Analysis

To evaluate the structural and functional features of *ZmVOZ* proteins, the ExPASy ProtParam tool (https://web.expasy.org/protparam/) was used to calculate molecular weight, isoelectric point (pI), aliphatic index, instability index, and GRAVY (Grand Average of Hydropathy).

Conserved domain analysis was conducted through the NCBI Conserved Domain Database (CDD) and InterProScan to confirm the presence of *VOZ*-specific domains, notably the zinc finger-like domain (*VOZ*) and possible coiled-coil regions. Subcellular localization was predicted using CELLO v2.5 (cello.life.nctu.edu.tw) to assess the nuclear or cytoplasmic localization of *ZmVOZ* proteins.

## 3.6 Motif Composition Analysis of the *ZmVOZ* Gene Family

Conserved motifs across *ZmVOZ* proteins were identified using MEME Suite v5.1.1 (https://meme-suite.org/tools/meme). The maximum number of motifs was set to 9 to ensure high specificity and reduce redundancy. Motifs were cross-compared with known domains using Pfam and SMART databases to correlate with functional significance. The analysis highlighted conserved regulatory or DNA-binding motifs critical to *VOZ*-mediated transcriptional regulation.

The analysis highlighted conserved regulatory or DNA-binding motifs critical to *VOZ*-mediated transcriptional regulation.

## 3.7 Phylogenetic Analysis of *ZmVOZ* Genes

A phylogenetic tree was constructed to explore evolutionary relationships among *ZmVOZ* genes and *VOZ* homologs from other plant species, including *Arabidopsis thaliana*, *Oryza sativa*, and *Glycine max*.

Multiple sequence alignments were performed using Clustal Omega, and the phylogenetic tree was visualized using the Interactive Tree Of Life (iTOL) tool (itol.embl). The resulting dendrogram elucidated the divergence and potential functional conservation between monocot (maize) and dicot VOZ genes.

## 3.8 Gene Structure and Motif Organization of *ZmVOZ* Genes

The exon-intron organization of *ZmVOZ* genes was analyzed using the Gene Structure Display Server (GSDS: http://gsds.gao-lab.org). Both genomic DNA and CDS sequences were aligned to map introns, exons, and untranslated regions (UTRs).

This analysis provided insights into the evolutionary conservation and structural variability within the gene family and supported the identification of gene duplication or structural divergence events.

## 3.9 Protein-Protein Interaction Network of *ZmVOZs*

Protein interaction networks were constructed for *ZmVOZ* proteins using STRING v12 (https://string-db.org/), with *Zea mays* as the organism. The interaction networks included predicted and experimentally validated protein partners, highlighting potential functional roles in transcriptional regulation, stress signaling, and developmental processes. The network visualizations, including color-coded nodes and edges, facilitated comparative analysis with known *AtVOZ* interaction partners to infer conserved pathways.

## 3.10 Promoter Analysis of *ZmVOZ* Genes

To investigate cis-regulatory elements in the promoter regions of *ZmVOZ* genes, 2000 bp upstream sequences from the transcription start site (TSS) were extracted using the Ensembl Plants *zea Mays* browser. These promoter sequences were analyzed using the PlantCARE database (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) to identify stress- and hormone-responsive elements, such as ABRE, MBS, TATA-box, and G-box motifs. The presence of these elements provided insights into the transcriptional regulation and potential environmental responsiveness of *ZmVOZ* genes

# Chapter 4

# Results

## 4.1 *Zea mays*-Wide Identification and Analysis of *VOZ* Genes in *Zea mays*

In the present study, *Zea mays*-wide identification and analysis of the *VOZ* (Vascular plant One-Zinc finger) gene family in *Zea mays* was carried out using well-characterized *VOZ* sequences from *Arabidopsis thaliana* as reference. Initially, full-length protein-coding sequences of *Arabidopsis thaliana VOZ* genes were obtained from The *Arabidopsis* Information Resource (TAIR: www.arabidopsis.org) using their respective gene accession numbers. Among multiple *VOZ* variants, the longest sequence was selected as a representative query for downstream analysis.

The selected *AtVOZ* protein sequence was then used as a query in a BLASTP search against the *Zea mays* in the NCBI Database (www.ncbi.nlm.nih.gov/zea Mays/gdv/). The BLAST results were retrieved and exported in CSV format, containing protein descriptions, accession numbers, and alignment scores. These results were compiled into an Excel spreadsheet where each entry was assigned a unique Lab ID along with its accession number and gene description.

To refine the dataset, all retrieved sequences were aligned against each other to create a pairwise similarity matrix using Clustal Omega. This alignment allowed for the identification and removal of spliced isoforms, redundant sequences, and incomplete protein fragments. Only sequences with full-length conserved domains and minimal redundancy were retained for further analysis. Domain integrity was confirmed through the NCBI Conserved Domain Database (CDD) and Pfam identifiers.

Following this filtering process, **two high-confidence *VOZ* protein sequences from *Zea mays*** were identified and selected for further functional and structural characterization. These were:

1. *ZmVOZ1* - Accession No.: XP_035817390.1
2. *ZmVOZ2* - Accession No.: XP_001105277.2

The finalized protein sequences of *ZmVOZ1* and *ZmVOZ2* were downloaded in FASTA format. A separate Excel sheet was created to catalog the selected *VOZ* genes, including their Lab IDs, gene names, accession numbers, and sequence lengths. These curated entries served as the basis for subsequent analyses involving physicochemical characterization, domain annotation, motif identification, gene structure analysis, interaction networks, and evolutionary relationships.

*VOZ* gene identification in *Zea mays* was thus completed through a comparative genomics pipeline utilizing both homology-based searching with *Arabidopsis thaliana VOZ* sequences and domain-based confirmation via Pfam domain accessions.

## 4.2 Physicochemical Properties and Conserved Domains of *Zea mays*

Accession numbers of identical or redundant sequences retrieved from multiple databases were carefully cross-examined and documented to avoid duplication in the dataset. The final set of non-redundant *VOZ* sequences from *Zea mays* (*ZmVOZ1* and *ZmVOZ2*) was selected based on the presence of conserved functional domains. Domain validation was performed using the Conserved Domain

Database (CDD) provided by NCBI (www.ncbi.nlm.nih.gov/Structure/cdd.html). All candidate sequences, including the Arabidopsis thaliana *VOZ* reference sequences, were submitted to CD-Search in batch mode. Conserved domain hits were identified, and the results—comprising the query ID, domain hit type, domain position, E-value, bit-score, accession number, and short name—are summarized in Table 4.2.

Subsequently, the physicochemical properties of both *A. thaliana VOZ* proteins (*AtVOZs*) and the identified maize *VOZ* proteins (*ZmVOZ1* and *ZmVOZ2*) were analyzed using the ExPASy ProtParam tool (https://web.expasy.org/protparam/). The calculated features included protein molecular weight (MW), theoretical isoelectric point (pI), and the GRAVY (Grand Average of Hydropathicity) index. These results are compiled in Table 4.3.

The gene and protein characteristics of *ZmVOZ1* and *ZmVOZ2* showed close resemblance to those of their *Arabidopsis* counterparts. Both *Zea mays* and *Arabidopsis VOZ* proteins exhibited negative GRAVY values, suggesting that they are hydrophilic in nature. The theoretical pI values for all *VOZ* proteins in both species were found to be below 7, classifying them as acidic proteins. Moreover, the subcellular localization of all *VOZ* proteins was predicted using CELLO v2.5, and results indicated a predominant localization within the chloroplast, consistent across both *Zea mays* and *A. thaliana.*

The average protein lengths were calculated as follows: 486 amino acids for *AtVOZs*, 614 amino acids for *ZmVOZ1*, and 604 amino acids for *ZmVOZ2.* While the protein lengths were generally similar, a significant difference was observed in the gene lengths between the two species, possibly due to variations in intron-exon structures or untranslated regions.

TABLE 4.1: Conserved domains of *VOZ* gene family of *ZmVOZ1*, *ZmVOZ2*

| **Org** | **Query** | **Hit Type** | **Position** | **E-value** | **Bit score** | **Accession** | **Short Name** |
|---|---|---|---|---|---|---|---|
| *Zea mays* | Q#3 →ZmVOZ1 | super family | 413–489 | 0.00597536 | 37.189 | cl03558 | NAM Super family |

**Continued table from previous page**

| Org | Query | Hit Type | Position | E-value | Bit score | Accession | Short Name |
|---|---|---|---|---|---|---|---|
| | Q#4 →ZmVOZ2 | super family | 353–441 | 0.00156828 | 38.7298 | clo3558 | NAM Super family |

Table 4.2: Physicochemical properties of *AtVOZ1*, *AtVOZ2*, *ZmVOZ1* and *ZmVOZ2*

| Plant | Gene Name | Chr | Position | Gene Length | Protein Length | Molecular Weight | Isoelectric Point | GRAVY | Location |
|---|---|---|---|---|---|---|---|---|---|
| Arabidopsis | *AtVOZ1* | 1 | 10029713 - 10031476 | 1764 | 486 | 54079.7 | 5.73 | -0.636 | Nucleus |
| | *AtVOZ2* | 1 | 10029713 - 10031476 | | 486 | 54079.7 | 5.73 | -0.636 | Nucleus |
| Corn | *ZmVOZ1* | 8 | 180764676 - 180766378 | 1702 | 614 | 68466.39 | 5.6 | -0.764 | Nucleus |
| | *ZmVOZ2* | 6 | 170389522 - 170392419 | 2898 | 604 | 66355.24 | 5.74 | -0.737 | Nucleus |

## 4.3 Gene Structure Determination

The gene and coding sequences (CDS) of *Arabidopsis thaliana VOZ* genes (*AtVOZ1* and *AtVOZ2*) and *Zea mays VOZ* genes (*ZmVOZ1* and *ZmVOZ2*) were analyzed to investigate their structural features, including the arrangement of exons, introns, and untranslated regions (UTRs).

This analysis was carried out using the Gene Structure Display Server (GSDS), an online tool that graphically illustrates gene structures based on input genomic and CDS sequences.

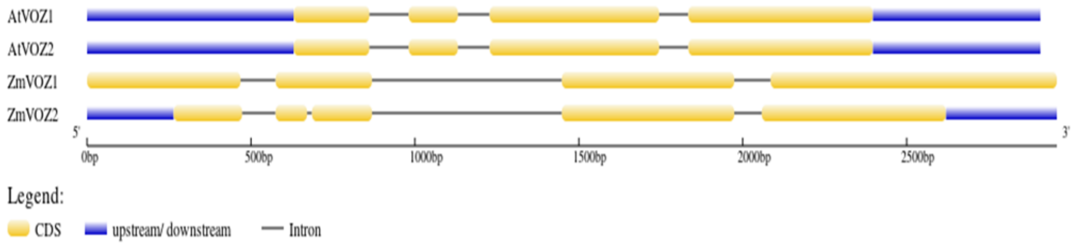


FIGURE 4.1: Gene Structure

The results revealed a conserved gene structure within each species, but notable differences between species:

In *Arabidopsis thaliana*, both *AtVOZ1* and *AtVOZ2* contained four exons (CDS regions), which were interrupted by three introns. These genes also included clearly defined 5' and 3' UTRs, shown in blue, suggesting the presence of regulatory elements at both ends. The intron lengths and positions were consistent, indicating a high level of structural conservation between the two *AtVOZ* genes.

In contrast, *Zea mays VOZ* genes (*ZmVOZ1* and *ZmVOZ2*) had a simpler exon-intron structure, each consisting of three coding regions separated by introns. While the overall gene lengths appeared longer than their Arabidopsis counterparts, this was largely due to extended intron or UTR regions. *ZmVOZ2*, in particular, displayed a longer 3' UTR, which may reflect species-specific regulatory variation.

Despite the differences in exon count and intron distribution, the relative arrangement of coding regions was conserved, implying functional conservation of *VOZ* genes across monocots and dicots. However, the variation in UTR length and intron spacing may suggest divergence in gene regulation and expression patterns.

## 4.4 Protein-protein Interactions

To gain a deeper understanding of the functional significance of *VOZ* (Vascular plant One-Zinc finger) transcription factors in plants, a comprehensive protein-protein interaction (PPI) analysis was performed for *AtVOZ1* and *AtVOZ2* from

*Arabidopsis thaliana*, as well as *ZmVOZ1* and *ZmVOZ2* from *Zea mays*.

This analysis aimed to identify both known and predicted interacting partners to uncover the broader biological networks in which these transcription factors operate. The interaction profiles revealed that *AtVOZ1* and *AtVOZ2* engage with a shared set of regulatory proteins, including those involved in light signaling, cell cycle regulation, DNA repair, and nutrient response, highlighting their potential roles in developmental and environmental response pathways.

In contrast, the interaction networks of *ZmVOZ1* and *ZmVOZ2* were more distinct, with *ZmVOZ1* associating predominantly with stress- and pathogen-response proteins, while *ZmVOZ2* was linked to transcription factors and membrane-associated proteins involved in hormonal and metabolic regulation. These findings suggest that while *VOZ* proteins are structurally conserved, they may have undergone functional diversification across species to meet specific physiological and environmental demands.

### 4.4.1 *AtVOZ1*

The PPI network for *AtVOZ1* demonstrates its interactions with several key proteins involved in various cellular processes. Notably, PHYB (phytochrome B) suggests *AtVOZ1*'s role in light perception and signaling pathways. AVP1, a vacuolar proton pump, indicates involvement in ion transport and stress adaptation. CKB1 (Casein Kinase II beta subunit) implies that *AtVOZ1* may be involved in phosphorylation-mediated signaling cascades.

The presence of MAD1 reflects a potential role in mitotic checkpoint control, while T20H2.11 and IBI1 point to DNA repair and translation regulation, respectively. PHL3, a transcription factor associated with phosphate starvation response, suggests a role in nutrient signaling. Interactions with metabolic proteins like HISN6B, ARD4, and F18O22.30 further imply *AtVOZ1*'s contribution to amino acid biosynthesis, redox homeostasis, and primary metabolism. Overall, *AtVOZ1* appears to function at the intersection of developmental regulation, environmental response, and cellular homeostasis.

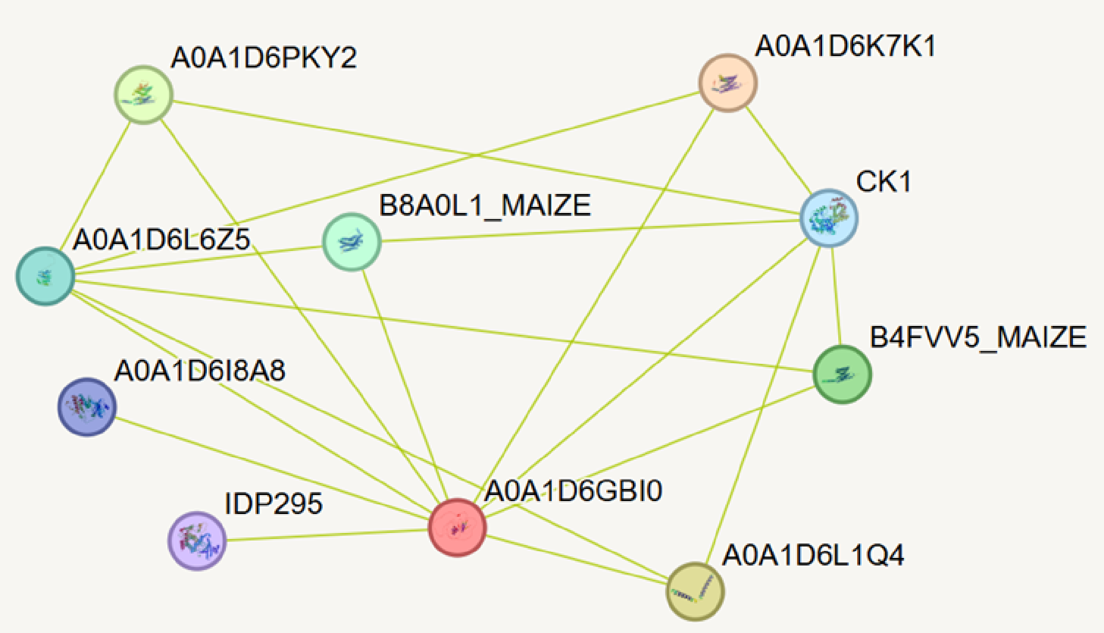


FIGURE 4.2: Protein-Protein Interaction of *AtVOZ1*

TABLE 4.3: Predicted functional partners of *AtVOZ1*

| | **Predicted functional partners of *AtVOZ1*** |
|---|---|
| PHYB | Phytochrome B; Regulatory photoreceptor which exists in two forms that are reversibly interconvertible by light: the Pr form |
| AVP1 | Pyrophosphate-energized vacuolar membrane proton pump 1; Contributes to the transtonoplast (from cytosol to vacuole |
| CKB1 | Casein kinase II subunit beta-1; Plays a complex role in regulating the basal catalytic activity of the alpha subunit. |
| T20H2.11 | DNA ligase-like protein. |
| MAD1 | Mitotic spindle checkpoint protein MAD1; Required for the execution of the mitotic checkpoint which monitors the process |
| HISN6B | Histidinol-phosphate aminotransferase 2, chloroplastic; Belongs to the class-II pyridoxal-phosphate-dependent aminotransf |
| F18O22.30 | Thioredoxin superfamily protein |
| ARD4 | 1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 4; Catalyzes the formation of formate and 2-keto-4- methylthiobutyrat |
| IBI1 | Aspartate–tRNA ligase 2, cytoplasmic; Catalyzes the specific attachment of an amino acid to its cognate tRNA in a 2 step |
| PHL3 | Protein PHR1-LIKE 3; Transcriptional activator. Probable component of the central regulatory system controlling transcriptional responses to Pi starvation. Binds in a sequence- specific manner to phosphate starvation-regulated promoters. Required for female gametophyte development and function. |

### 4.4.2 ***AtVOZ2***

The PPI network for *AtVOZ2* reveals a very similar interaction pattern to *AtVOZ1*, supporting the idea that these two transcription factors may share overlapping or redundant functions in Arabidopsis thaliana. *AtVOZ2* also interacts with PHYB, suggesting a conserved role in light-mediated developmental processes. Its association with AVP1, CKB1, and MAD1 points to functions in vacuolar transport, protein phosphorylation, and cell cycle control.

Its association with AVP1, CKB1, and MAD1 points to functions in vacuolar transport, protein phosphorylation, and cell cycle control. Additional links to PHL3, ARD4, T20H2.11, IBI1, HISN6B, and F18O22.30 highlight *AtVOZ2*'s involvement in transcription regulation, DNA repair, amino acid metabolism, and stress signaling.

The consistency of these interactions with those seen in the *AtVOZ1* network indicates that *AtVOZ2* may participate in co-regulation of growth, stress adaptation, and metabolic balance, likely acting within the same or closely related pathways.

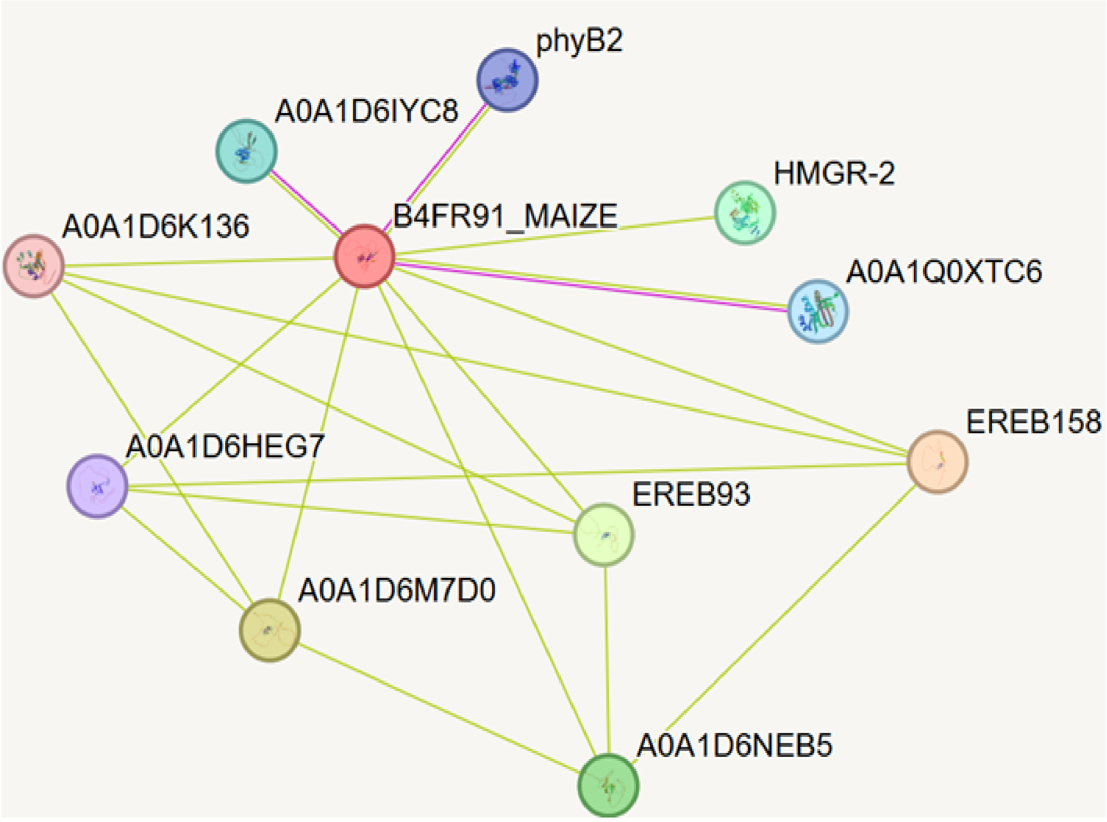


FIGURE 4.3: Protein-Protein Interaction of *AtVOZ2*

Table 4.4: Predicted functional partners of *AtVOZ2*

| | **Predicted functional partners of *AtVOZ1*** |
|---|---|
| PHYB | Phytochrome B; Regulatory photoreceptor which exists in two forms that are reversibly interconvertible by light: the Pr form |
| AVP1 | Pyrophosphate-energized vacuolar membrane proton pump 1; Contributes to the transtonoplast (from cytosol to vacuole |
| CKB1 | Casein kinase II subunit beta-1; Plays a complex role in regulating the basal catalytic activity of the alpha subunit. |
| T20H2.11 | DNA ligase-like protein. |
| MAD1 | Mitotic spindle checkpoint protein MAD1; Required for the execution of the mitotic checkpoint which monitors the process |
| HISN6B | Histidinol-phosphate aminotransferase 2, chloroplastic; Belongs to the class-II pyridoxal-phosphate-dependent aminotransf |
| F18O22.30 | Thioredoxin superfamily protein |
| ARD4 | 1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 4; Catalyzes the formation of formate and 2-keto-4- methylthiobutyrat |
| IBI1 | Aspartate–tRNA ligase 2, cytoplasmic; Catalyzes the specific attachment of an amino acid to its cognate tRNA in a 2 step |
| PHL3 | Protein PHR1-LIKE 3; Transcriptional activator. Probable component of the central regulatory system controlling transcriptional responses to Pi starvation. Binds in a sequence- specific manner to phosphate starvation-regulated promoters. Required for female gametophyte development and function. |

### 4.4.3 *ZmVOZ1*

It illustrates a protein-protein interaction (PPI) network centered around the *VOZ1* protein, showing its direct and indirect interactions with various other proteins such as PHYB, AVP1, CKB1, and others.

Each node represents a protein, and the connecting lines indicate experimentally determined or predicted interactions, which are essential for understanding the functional associations and regulatory pathways involving *VOZ1* in the biological system.

Each node represents a protein, and the connecting lines indicate experimentally determined or predicted interactions, which are essential for understanding the functional associations and regulatory pathways involving *VOZ1* in the biological system.

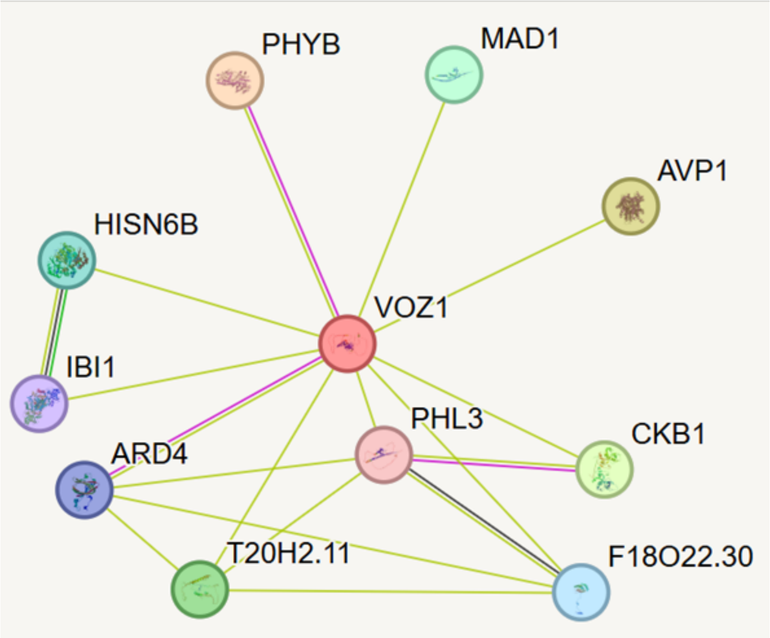


FIGURE 4.4: Protein-Protein Interaction of *ZmVOZ1*

TABLE 4.5: Predicted functional partners of *ZmVOZ1*

| | **Predicted functional partners of *ZmVOZ1*** |
|---|---|
| A0A1D6K7K1 | Tobamovirus multiplication protein 3. |
| A0A1D6L1Q4 | Tobamovirus multiplication protein 3 |
| A0A1D6PKY2 | Tobamovirus multiplication protein 3. |
| B4FVV5_MAIZE | Tobamovirus multiplication protein 3 |
| B8A0L1_MAIZE | Tobamovirus multiplication protein 3. |
| A0A1D6L6Z5 | Calcium-dependent protein kinase 1. |
| CK1 | Calcium dependent protein kinase4. |
| A0A1D6I8A8 | Chitin elicitor receptor kinase 1. |
| IDP295 | Chitin elicitor receptor kinase 1. |

### 4.4.4 *ZmVOZ2*

The figure presents a similar PPI network, but with D7KCX7_ARALL as the central protein. This network highlights the interactions of D7KCX7_ARALL with several other proteins, including AVP1 and PHYB, as well as uniquely labeled proteins like D7MHW6_ARALL and D7KF19_ARALL. The visualization helps to map out the molecular relationships and potential signaling pathways in which D7KCX7_ARALL is involved, offering insights into its biological roles and connectivity within the cellular context.

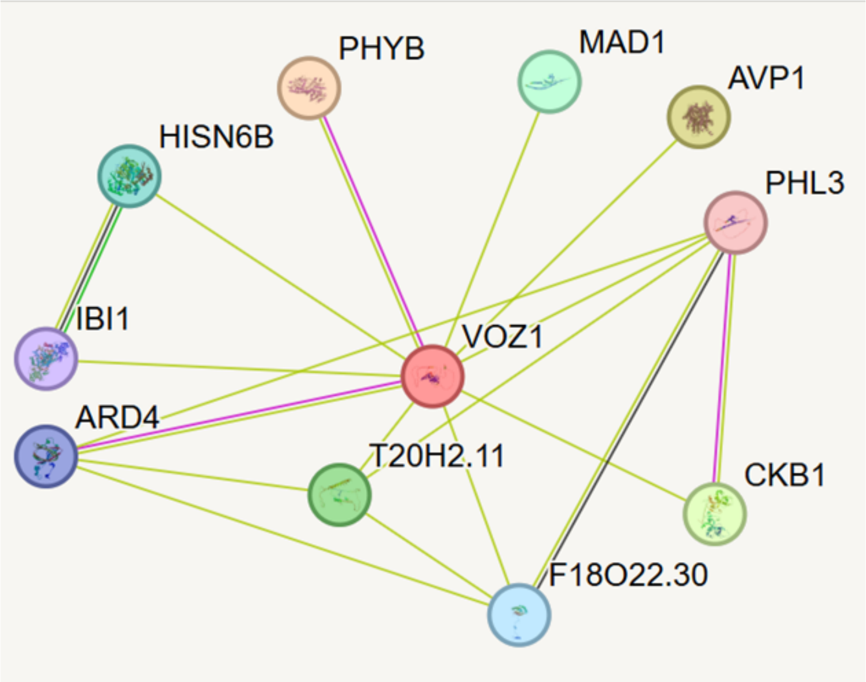


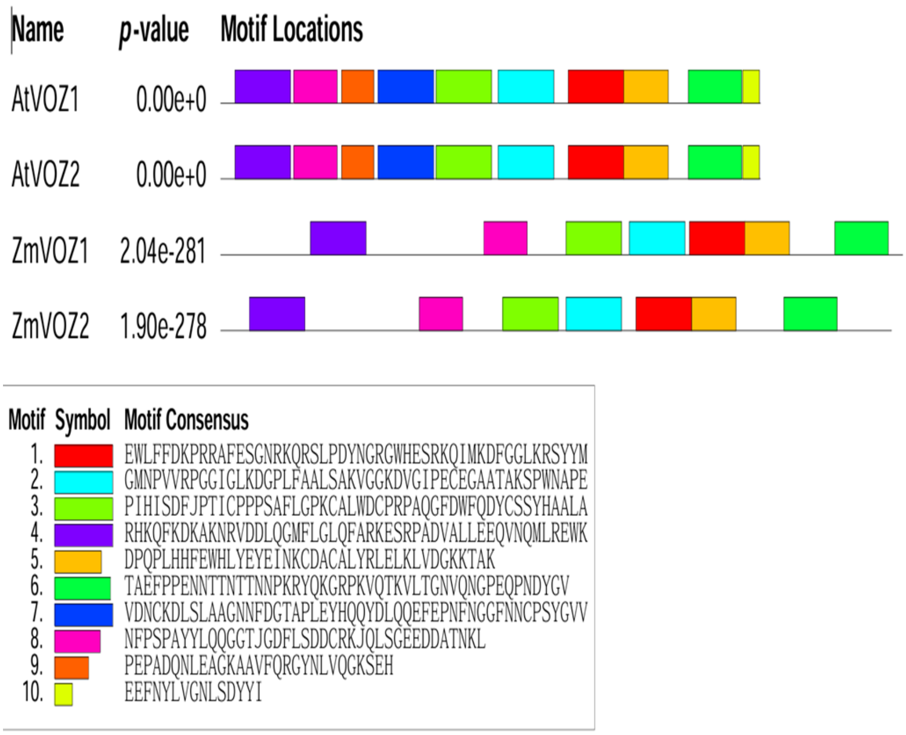
FIGURE 4.5: Protein-Protein Interaction of *ZmVOZ2*

TABLE 4.6: Predicted functional partners of *ZmVOZ2*

| | **Predicted functional partners of *ZmVOZ2*** |
|---|---|
| EREB158 | Ethylene-responsive transcription factor 12 |
| A0A1D6M7D0 | Putative AP2/EREBP transcription factor superfamily protein |
| EREB93 | Ethylene-responsive transcription factor 12. |
| A0A1D6NEB5 | MYB9. |
| HMGR-2 | 3-hydroxy-3-methylglutaryl-coenzyme A reductase 2 |
| A0A1Q0XTC6 | Phytochrome B. |
| A0A1D6IYC8 | Phytochrome B. |
| phyB2 | Phytochrome; Regulatory photoreceptor which exists in two forms that are reversibly interconvertible by light: the Pr form |
| A0A1D6HEG7 | Membrane steroid-binding protein 1. |
| A0A1D6K136 | Membrane steroid-binding protein 1; Belongs to the cytochrome b5 family. |

## 4.5 Consensus Motifs Composition in *AtVOZs* and *ZmVOZs*

To investigate conserved functional regions within *VOZ* transcription factors, protein sequences of *AtVOZ1*, *AtVOZ2* (*Arabidopsis thaliana*), *ZmVOZ1*, and *ZmVOZ2* (Zea mays) were analyzed for the presence of consensus motifs. A total of 10 conserved motifs were identified using MEME Suite, as shown in Figure 4.6. The analysis revealed that several motifs were highly conserved across both species, suggesting that these regions are critical for the structural integrity and functional activity of VOZ proteins. The analysis revealed that several motifs were highly conserved across both species, suggesting that these regions are critical for the structural integrity and functional activity of VOZ proteins. While the overall motif architecture was largely similar, slight variations in motif arrangement and length were observed between *Arabidopsis* and maize sequences, indicating possible functional divergence or species-specific regulatory adaptations. These conserved motifs are likely associated with DNA-binding, transcriptional regulation, and stress response functions that are typical of the VOZ protein family.



| Motif | Symbol | Motif Consensus |
|---|---|---|
| 1. | 🟥 | EWLFFDKPRRAFESGNRKQRSLPDYNGRGWHESRKQIMKDFGGLKRSYYM |
| 2. | 🟦 | GMNPVVRPGGIGLKDGPLFAALSAKVGGKDVGIPECEGAATAKSPWNAPE |
| 3. | 🟩 | PIHISDFJPTICPPPSAFLGPKCALWDCPRPAQGFDWFQDYCSSYHAALA |
| 4. | 🟪 | RHKQFKDKAKNRVDDLQGMFLGLQFARKESRPADVALLEEQVNQMLREWK |
| 5. | 🟨 | DPQPLHHFEWHLYEYEINKCDACALYRLELKLVDGKKTAK |
| 6. | 🟩 | TAEFPPENNTTNTTNNPKRYQKGRPKVQTKVLTGNVQNGPEQPNDYGV |
| 7. | 🟦 | VDNCKDLSLAAGNNFDGTAPLEYHQQYDLQQEFEPNFNGGFNNCPSYGVV |
| 8. | 🟪 | NFPSPAYYLQQGGTJGDFLSDDCRKJQLSGEEDDATNKL |
| 9. | 🟧 | PEPADQNLEAGKAAVFQRGYNLVQGKSEH |
| 10. | 🟨 | EEFNYLVGNLSDYYI |

FIGURE 4.6: Consensus motifs of *AtVOZs* and *ZmVOZs*

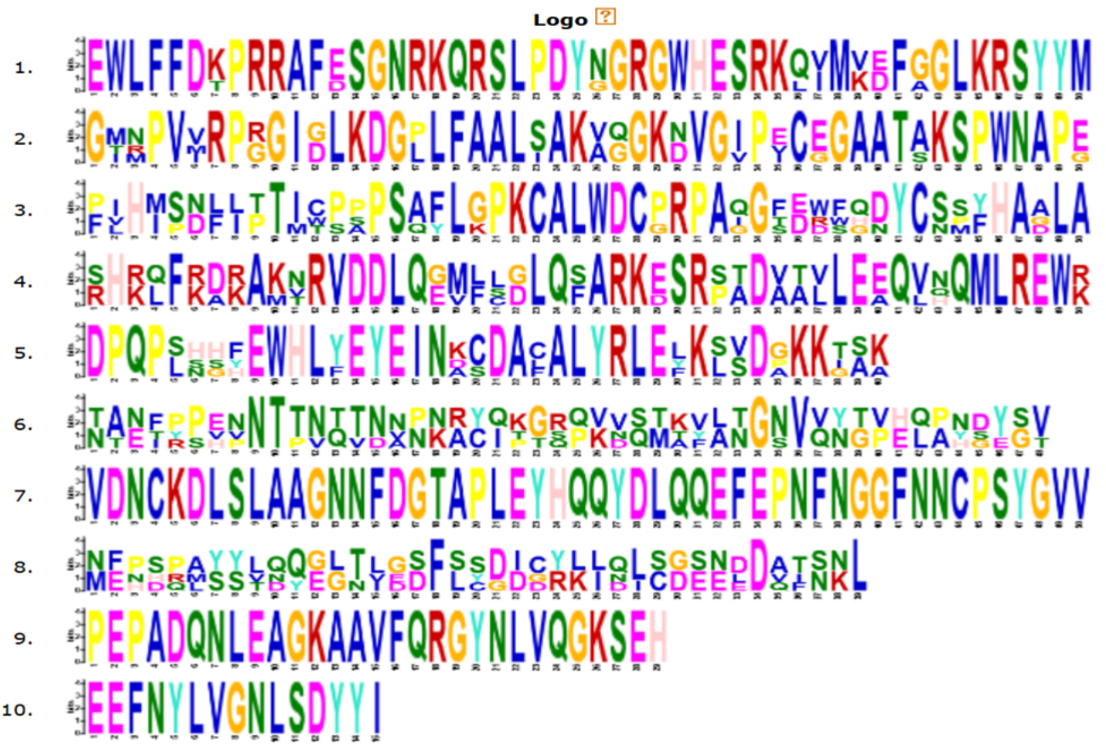


FIGURE 4.7: Logos of identified motifs

## 4.6 Sequence Alignment and Phylogenetic Relationship of *VOZ* Gene Family

The evolutionary relationship of *VOZ* (Vascular plant One Zinc-finger) genes among different plant species, including *Zea mays* (maize) and *Arabidopsis thaliana*, can be elucidated through phylogenetic tree analysis. Sequences from various strains were aligned and analyzed using the neighbor-joining method, with the tree constructed via CLUSTAL OMEGA and visualized using iTOL. This approach allows for the identification of evolutionary divergence and gene family expansion across species.

A phylogenetic tree visually represents the evolutionary relationships among *VOZ* genes from different species. Major clades indicate groups of genes that have evolved from common ancestors. The branching patterns show how gene duplication, divergence, and lineage-specific expansion have shaped the *VOZ* gene family in both *Zea mays* and *Arabidopsis thaliana*. The separation of maize *VOZ* genes into distinct clades from *Arabidopsis VOZ* genes suggests both ancient and recent evolutionary events, highlighting the dynamic history of this gene family in plants

This approach allows for the identification of evolutionary divergence and gene family expansion across species.

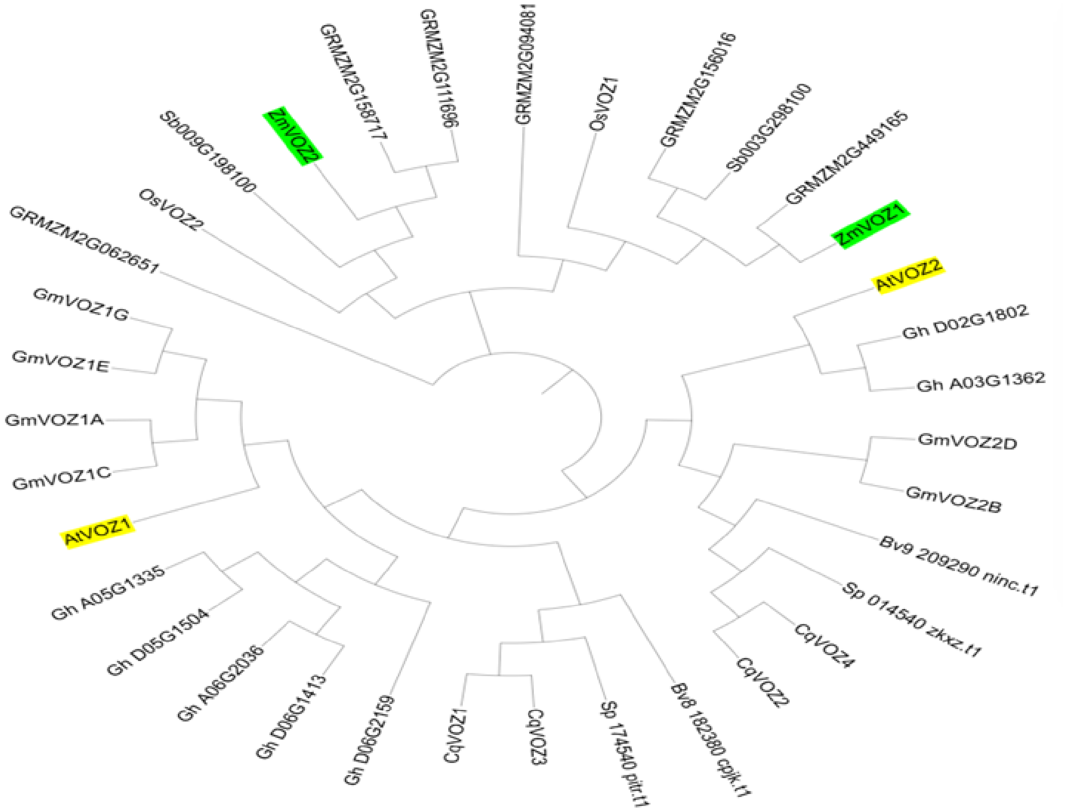


FIGURE 4.8: Phylogenetic tree highlighting evolutionary relationship of *Arabidopsis thaliana* and *Zea mays*

### 4.6.1 Structure of the Phylogenetic Tree

The phylogenetic tree of *VOZ* genes is typically divided into four major clades based on branching patterns and sequence grouping.

*VOZ* genes from *Zea mays* (*ZmVOZ*) and *Arabidopsis thaliana* (*AtVOZ*) are distributed across these clades, indicating both conservation and divergence within the gene family.

In *Zea mays*, there are more *VOZ* gene members compared to *Arabidopsis*, reflecting gene family expansion in monocots like maize.

### 4.6.2 Clade Distribution and Evolutionary Insight

Each clade represents a group of *VOZ* genes that share a closer evolutionary relationship. For example, *ZmVOZ1* and *ZmVOZ2* may be found in different clades, suggesting significant divergence and potentially distinct functional roles.

The *VOZ* gene family in *Zea mays* is more expanded than in *Arabidopsis*, with maize containing up to six *VOZ* genes, while *Arabidopsis* typically has two (*AtVOZ1* and *AtVOZ2*).

The tree shows that monocot *VOZ* genes (like those from *Zea mays*) and dicot *VOZ* genes (like those from *Arabidopsis*) are grouped into separate but sometimes closely related clades, reflecting the evolutionary split between monocots and dicots.

Some clades contain genes exclusively from monocots or dicots, while others may show mixed grouping, indicating ancient gene duplication events followed by lineage-specific expansions or losses.

### 4.6.3 Functional Implications

Divergence of *VOZ* genes into separate clades suggests that these genes may have evolved to acquire specialized functions in different plant lineages.

For example, the placement of *ZmVOZ1* in one clade and *ZmVOZ2* in another could indicate functional specialization, such as differential roles in stress response, flowering, or development.

The clustering of *VOZ* genes from *Zea mays* with those from other monocots, and those from Arabidopsis with other dicots, supports the idea that these genes have retained lineage-specific functions while also sharing some conserved roles across plant species

## 4.7 Promoter Analysis

Promoter analysis serves as a critical approach for understanding the regulatory mechanisms and potential biological roles of genes. In this study, the 2000 bp upstream promoter regions of *AtVOZ1*, *AtVOZ2* (*Arabidopsis thaliana*) and *ZmVOZ1*, *ZmVOZ2* (*Zea mays*) were analyzed to identify cis-regulatory elements that may influence their expression. The identified elements were categorized into three functional groups: phytohormone-responsive (PR), plant growth and development-related (PGD), and stress-responsive (SR) elements. These cis - elements provide insights into the possible involvement of *VOZ* genes in various hormonal signaling pathways (such as ABA, GA, and auxin), developmental processes, and biotic or abiotic stress responses. The distribution and abundance of these elements varied across the four promoters, suggesting that species-specific and gene-specific regulatory patterns may influence *VOZ* gene expression. The detailed classification and frequency of these elements are presented in Table 4.7.

TABLE 4.7: Number of cis-regulatory elements identified in promoter regions of AtVOZ,VOZ1 and VOZ2 Gene Families.

| | **Plant growth & Development** | | | **Stress Responsive** | | | | **Phytohormone Responsive** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene** | **Box4** | **O2-site** | **GCN4-motif** | **WUN Motif** | **ARE** | **MBS** | **GC-Motif** | **CGTCA-Motif** | **TGACG-Motif** | **Gare -motif** | **P-Box** | **ABRE** | **ERE** | **TGA-element** |
| AtVOZ | | | | 1 | | | | | 1 | 1 | | 2 | 1 | |
| VOZ1 | 1 | 1 | 1 | | 2 | 1 | 3 | 3 | 3 | 1 | 1 | 2 | | 1 |
| VOZ2 | 1 | 1 | 1 | | 2 | 1 | 3 | 3 | 3 | 1 | 1 | 2 | | 1 |

# Chapter 5

# Discussion

The present study provides an in-depth *Zea mays*-wide characterization of the *VOZ* (*Vascular plant One-Zinc finger*) gene family in *Zea mays* (maize), comparing it with its well-studied homologs in *Arabidopsis thaliana*. Using Arabidopsis *VOZ* gene sequences as queries, we identified two high-confidence *VOZ* genes in maize *ZmVOZ1* and *ZmVOZ2* highlighting the conserved and divergent features of *VOZ* transcription factors in monocots and dicots. These maize *VOZ* genes were confirmed to harbor conserved NAM superfamily domains and acidic, hydrophilic physicochemical characteristics typical of nuclear-localized transcription factors [42] [30]. These conserved structural features suggest their preserved roles in transcriptional regulation across evolutionary lineages.

However, notable variations in gene length, exon-intron architecture, and untranslated regions (UTRs) between maize and *Arabidopsis* *VOZ* genes were observed. These structural discrepancies point toward evolutionary divergence in regulatory mechanisms, possibly reflecting species-specific adaptation [20][40]. Despite these differences, motif analysis using MEME Suite revealed that both species retained a core set of conserved motifs essential for DNA-binding and gene regulation [16] [30]. Such motif conservation suggests a fundamental functional requirement for *VOZ* transcription factors, even in the face of structural divergence.

The differences extended beyond gene structure. Protein-protein interaction network analysis uncovered that *Arabidopsis VOZ* proteins interact with light signaling components, flowering regulators, and nutrient sensing factors functions aligned with previously established roles in photoperiodic flowering and growth regulation [4][30]. Conversely, *ZmVOZ1* was predicted to interact with stress-related proteins such as chitin receptor kinases and calcium-dependent protein kinases (CDPKs), implicating it in biotic stress and calcium signaling pathways [12][29]. *ZmVOZ2* was linked to ethylene-responsive elements, MYB transcription factors, and membrane-associated proteins, indicating its role in hormone signaling and membrane-associated regulatory mechanisms [14] [23]. These differences hint at functional divergence or subfunctionalization of *VOZ* genes in maize to suit its unique environmental challenges and developmental programming.

Cis-element analysis of the *VOZ* promoters in both species further supported these findings. Promoter regions of *ZmVOZ1* and *ZmVOZ2* harbored a higher frequency of stress-responsive elements compared to those in Arabidopsis, including those responsive to abscisic acid, gibberellin, and auxin [15][31][32]. This enrichment suggests an active role of maize *VOZ* genes in coordinating abiotic stress responses and possibly hormonal crosstalk, thus enabling the plant to respond more effectively to environmental stressors such as drought, salinity, and heat [14][15].

Phylogenetic analysis placed *ZmVOZ1* and *ZmVOZ2* in distinct clades, separate not only from each other but also from *Arabidopsis VOZ* genes, indicating lineage-specific expansion possibly due to *zea Mays* duplication events common in maize and other monocots [16][49]. These lineage-specific expansions are known to drive gene neofunctionalization, where duplicated genes acquire new roles, often associated with environmental adaptation [16]. The diversification of *VOZ* gene functions in maize could thus be an evolutionary advantage, enhancing stress resilience and developmental plasticity in a crop of significant agronomic importance.

Taken together, the findings of this study suggest that although the *VOZ* gene family maintains a conserved core structure and function, significant divergence in sequence architecture, promoter elements, and interaction networks reflects their functional plasticity across plant lineages. This divergence likely underpins the observed species-specific adaptations of *VOZ* genes. The annotation and computational predictions presented here serve as a valuable foundation for future research aiming to elucidate the roles of *VOZ* genes in maize development and stress tolerance. Ultimately, these insights may contribute to targeted crop improvement strategies aimed at enhancing resilience under diverse environmental conditions.

# Chapter 6

# Conclusion

This study presents a comprehensive *Zea mays*-wide analysis and comparative characterization of VOZ transcription factors in *Zea mays*, employing *Arabidopsis thaliana* VOZs as a reference. Two *VOZ* genes *ZmVOZ1* and *ZmVOZ2* were identified, and their domain structure, physicochemical properties, motif composition, protein interactions, promoter elements, and evolutionary relationships were thoroughly analyzed. Domain analysis confirmed that both maize proteins belong to the NAM superfamily and possess features indicative of transcriptional regulators, including nuclear localization, hydrophilicity, and acidic character [42]. Although coding sequences were conserved, differences in gene structure and promoter architecture suggest evolutionary divergence with potential impacts on gene regulation.

Functional motif analysis demonstrated conserved sequence patterns across species, implying preservation of critical DNA-binding domains. However, protein–protein interaction analysis revealed that while *Arabidopsis VOZ* proteins are associated with development and photoperiodic signaling, their maize counterparts interact with stress-related and hormone-responsive proteins, suggesting functional diversification [12][29], [23]. Promoter analysis corroborated these patterns, with maize *VOZ* genes exhibiting a higher frequency of cis-elements related to stress and hormonal pathways, further supporting their role in environmental response [14][15][32].

Collectively, these data underscore the conserved nature of *VOZ* genes in structural and sequence domains but highlight species-specific differences in regulatory elements and functional networks. This dual nature of conservation and divergence enhances our understanding of transcription factor evolution and paves the way for translational research aimed at developing stress-resilient crops like maize.

## 6.1 Future Directions

To advance the understanding of *VOZ* gene functions in *Zea mays*, future research should adopt both experimental and computational approaches. One key direction involves expression profiling of ZmVOZ1 and *ZmVOZ2* under a variety of abiotic and biotic stresses such as drought, salinity, heat, cold, and pathogen infection. Using tools like qRT-PCR, RNA-seq, or microarrays will help identify stress-specific and developmentally regulated expression patterns [10][35][49]. Additionally, tissue-specific expression studies can provide insight into organ-level gene function, determining whether *VOZ* activity is prominent in roots, shoots, or reproductive tissues.

Another crucial step is functional validation through genetic manipulation. Techniques such as CRISPR/Cas9-based gene knockouts and overexpression lines will reveal the phenotypic consequences of altering *VOZ* gene function. For instance, loss-of-function mutants can shed light on *VOZ* roles in flowering, seed germination, or stress response, while heterologous expression in Arabidopsis may help confirm conserved or divergent functions [4], [50].

Furthermore, elucidating the subcellular localization of *VOZ* proteins via GFP-fusion constructs in maize protoplasts or transgenic lines will help confirm their nuclear or cytoplasmic dynamics under different stress conditions [29][42]. This should be complemented by target gene discovery through methods like chromatin immunoprecipitation sequencing (ChIP-seq), DNA affinity purification sequencing (DAP-seq), or yeast one-hybrid (Y1H) assays to identify direct DNA-binding sites and downstream transcriptional targets [21].

Equally important are interactome studies, including yeast two-hybrid screening, co-immunoprecipitation, and LC-MS/MS-based proteomics, to map *VOZ* interaction networks and identify co-regulators involved in stress signaling and development [12][23]. Lastly, expanding the phylogenetic framework by including *VOZ* homologs from other monocots and dicots such as rice, wheat, barley, and soybean will help elucidate the evolutionary trajectory and functional diversification of *VOZ* genes across plant species [16], [49].

In the long term, the integration of these findings into crop improvement strategies particularly for enhancing stress resilience and yield stability in maize could have significant agricultural and economic benefits in the face of climate change.

# Bibliography

[1] Jeffrey Doebley, Ania Stec, and Charles Gustus, "Teosinte branched1 and the origin of maize: Evidence for epistasis and the evolution of dominance," Genetics, vol. 141, no. 1, pp. 333-346, 2024.

[2] Masanobu Matsuoka, Eunice C. Mitchell, Wei H. Hu, Junhua Jia, Jeffrey J. S. Cambron, and Jeffrey Ross-Ibarra, "A single domestication for maize shown by multilocus microsatellite genotyping," Proceedings of the National Academy of Sciences of the United States of America, vol. 99, no. 9, pp. 6080-6084, 2022.

[3] Robert A. Freeling and Doris Lisch, "How transposons silence genes and increase gene expression," Current Opinion in Plant Biology, vol. 9, no. 6, pp. 503-510, 2020.

[4] Robert J. Lambert, "Maize kernel development and composition," Crop Science, vol. 42, no. 3, pp. 983-987, 2022.

[5] Food and Agriculture Organization (FAO), Maize in human nutrition, FAO Food and Nutrition Series, no. 25, 2020.

[6] U.S. Department of Agriculture, World Agricultural Supply and Demand Estimates, WASDE-627, 2023.

[7] Sanjay Chauhan, Rajesh Tyagi, and Naveen Kumar, "Corn: Chemistry and functional properties," Journal of Food Science and Technology, vol. 57, no. 10, pp. 3287-3299, 2020.

[8] Pakistan Agricultural Research Council, Annual Maize Report, Islamabad, 2023.

[9] Pakistan Bureau of Statistics, Agricultural Statistics of Pakistan 2022-23, Government of Pakistan, 2023.

[10] Abdul Khan, Muhammad Yousaf, Saeed Ahmed, and Muhammad Arshad, "Performance evaluation of maize hybrids in different agro-ecological zones of Pakistan," Pakistan Journal of Agricultural Sciences, vol. 58, no. 4, pp. 1121-1128, 2021.

[11] M. Yousaf et al., "Assessment of drought tolerance in maize genotypes under field conditions," Pak. J. Bot., vol. 54, no. 2, pp. 421-428, 2022.

[12] A. Raza et al., "Impact of hybrid maize promotion strategies on productivity in Pakistan," J. Rural Dev. Agric., vol. 5, no. 2, pp. 87-94, 2020.

[13] B. McClintock, "The significance of responses of the genome to challenge," Science, vol. 226, no. 4676, pp. 792-801, 1984.

[14] MaizeGDB: Maize Genetics and Genomics Database. . Available: https://www.maizegdb.org

[15] S. Anderson et al., "Functional genomics of maize: Advances and prospects," Plant Cell Rep., vol. 40, no. 2, pp. 235-250, 2021.

[16] J. Yamasaki, T. Takagi, and Y. Hashimoto, "VOZ1 and VOZ2: Zinc finger proteins involved in stress regulation in Arabidopsis," Plant Cell Physiol., vol. 44, no. 5, pp. 556-563, 2003.

[17] A. Nakai et al., "Nuclear localization and transcriptional regulation by VOZ1 and VOZ2 in plants," Mol. Genet. Genomics, vol. 274, pp. 543-551, 2005.

[18] A. Mittal, S. Khurana, and P. Tyagi, "VOZ transcription factors: Modular regulators of abiotic stress tolerance," J. Plant Biochem. Biotechnol., vol. 27, pp. 123-131, 2018.

[19] M. Gao et al., "Comparative domain analysis and protein modeling of VOZ genes across angiosperms," Plant Sci., vol. 282, pp. 12-22, 2019.

[20] A. Kumar, R. Singh, and D. Sharma, "Mutant analysis of Arabidopsis VOZ genes reveals roles in flowering and drought response," Planta, vol. 250, pp. 243-254, 2019.

[21] C. Li et al., "Cross-talk between VOZ and DREB transcription factors during heat stress in plants," Plant Cell Rep., vol. 39, pp. 1345-1357, 2020.

[22] M. Koguchi et al., "VOZ1/VOZ2 regulate flowering and interact with FLC in Arabidopsis," Plant J., vol. 79, pp. 1062-1073, 2024.

[23] T. Yoshida and K. Ohama, "Stress-induced nuclear localization of VOZ proteins in Arabidopsis," Mol. Plant, vol. 9, pp. 565-567, 2021.

[24] Y. Wu et al., "Functional analysis of VOZ genes in rice under abiotic stress," Front. Plant Sci., vol. 11, p. 984, 2020.

[25] H. Liu et al., "VOZ transcription factors modulate nitrogen fixation in soybean," J. Exp. Bot., vol. 71, pp. 2230-2244, 2020.

[26] B. Zhang et al., "Characterization of VOZ family in wheat and tomato," Plant Cell Tissue Organ Cult., vol. 132, pp. 421-434, 2017.

[27] J. Chen, Y. Liu, and Z. Zhang, "Evolutionary dynamics of VOZ genes in monocots and dicots," Int. J. Mol. Sci., vol. 21, no. 5, p. 1675, 2020.

[28] R. Singh et al., "Comparative phylogenetic analysis of zinc finger transcription factors in plants," BMC Genomics, vol. 22, no. 1, p. 541, 2021.

[29] A. Rauf et al., "Transcriptome mining for zinc finger transcription factors in Zea mays: A preliminary overview," Pak. J. Bot., vol. 53, no. 2, pp. 623-631, 2021.

[30] L. Ali et al., "Abiotic stress-related transcription factors in corn: A review," Agron. J., vol. 113, no. 3, pp. 1701-1714, 2021.

[31] W. Hussain et al., "Functional genomics and genome editing for maize improvement," Plants, vol. 10, no. 8, p. 1622, 2021.

[32] B. Li et al., “Expression analyses of soybean VOZ transcription factors and the role of GmVOZ1G in drought and salt stress tolerance,” Int. J. Mol. Sci., vol. 21, no. 6, p. 2177, 2020.

[33] L. Liu, M. J. White, and T. H. MacRae, “Transcription factors and their genes in higher plants: functional domains, evolution and regulation,” Eur. J. Biochem., vol. 262, no. 2, pp. 247-257, 1999.

[34] Z. Liu et al., “Temporal transcriptome profiling reveals expression partitioning of homeologous genes contributing to heat and drought acclimation in wheat (Triticum aestivum L.),” BMC Plant Biol., vol. 15, p. 152, 2025.

[35] K. J. Livak and T. D. Schmittgen, “Analysis of relative gene expression data using real-time quantitative PCR and the 2-$\delta\delta$CT method,” Methods, vol. 25, pp. 402-408, 2021.

[36] O. Loudet et al., “A zinc knuckle protein that negatively controls morning-specific growth in Arabidopsis thaliana,” Proc. Natl. Acad. Sci. USA, vol. 105, no. 44, pp. 17193-17198, 2022.

[37] D. Luo et al., “VOZ1 and VOZ2 negatively regulate phytochrome B-mediated seed germination in Arabidopsis,” Biosci. Biotechnol. Biochem., vol. 84, no. 7, pp. 1384-1393, 2020.

[38] K. Madhu, A. Kaur, K. Singh, and S. K. Upadhyay, “Ascorbate oxidases in bread wheat: gene regulatory network, transcripts profiling, and interaction analyses,” Plant Growth Regul., vol. 103, pp. 209-224, 2024.

[39] K. Madhu, A. Sharma, A. Kaur, K. Singh, and S. K. Upadhyay, “Modulation in gene expression and enzyme activity suggests the role of monodehydroascorbate reductase in wheat,” Plant Sci., vol. 338, p. 111902, 2023.

[40] J. S. Mattick, “Introns: evolution and function,” Curr. Opin. Genet. Dev., vol. 4, pp. 823-831, 1994.

[41] T. Mitsis et al., “Transcription factors and evolution: an integral part of gene expression (Review),” World Acad. Sci. J., vol. 2, pp. 3-8, 2020.

[42] N. Mitsuda, T. Hisabori, K. Takeyasu, and M. H. Sato, “VOZ; isolation and characterization of novel vascular plant transcription factors with a one-zinc finger from Arabidopsis thaliana,” Plant Cell Physiol., vol. 7, pp. 845-854, 2004.

[43] Y. Nakai, S. Fujiwara, Y. Kubo, and M. H. Sato, “Overexpression of VOZ2 confers biotic stress tolerance but decreases abiotic stress resistance in Arabidopsis,” Plant Signal Behav., vol. 8, no. 3, p. e23358, 2013.

[44] P. V. Nazarov, A. Muller, and L. Vallar, “Co-expression analysis of large microarray data sets using coexpress software tool,” ResearchGate.net, 2010.

[45] D. A. Orozco-Nunnelly et al., “Pirin1 is a multifunctional protein that impacts light and UV responses in Arabidopsis,” PLoS ONE, vol. 9, no. 4, p. e93371, 2014.

[46] J. Paez-Valencia et al., “Plasma membrane localization of H+-PPase AVP1 in sieve element-companion cell complexes,” Plant Sci., vol. 181, pp. 23-30, 2021.

[47] I. Papatheodorou et al., “Expression atlas: gene and protein expression across multiple studies and organisms,” Nucleic Acids Res., vol. 46, no. D1, pp. D246-D251, 2020.

[48] U. J. Phukan, G. S. Jeena, and R. K. Shukla, “WRKY transcription factors: molecular regulation and stress responses in plants,” Front. Plant Sci., vol. 7, p. 760, 2022.

[49] L. Pingault et al., “Deep transcriptome sequencing provides new insights into the structural and functional organization of the wheat Zea mays,” Zea Mays Biol., vol. 16, no. 1, p. 29, 2020.

[50] K. V. S. K. Prasad, D. Xing, and A. S. N. Reddy, “VOZ transcription factors are positive regulators of salt tolerance in Arabidopsis,” Int. J. Mol. Sci., vol. 19, p. 3731, 2022.