

RESEARCH ARTICLE

IN-SILICO ANALYSIS OF HEAVY METALS STRESS-RESPONSIVE TRANSCRIPTION FACTORS IN PLANTS - A COMPREHENSIVE REVIEW

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Abstract

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In recent times, heavy metal contamination of the environment has become one of the major concerns. Various sources like industrial wastes, un-checked fertilizer application and other man-made causes have accelerated the spread of heavy metals in the environment. These are responsible for the disruption of plant homeostasis, altered gene expression and stunted growth in plants. Several genes and associated transcription factors have been found to play a key regulatory role in the uptake and detoxification of these heavy metal from commercially important crop plants. Transcription factors like WRKY, MYB, bHLH, bZIP have been reported to be of potential significance. With the advance of bioinformatics techniques, several in-silico tools like homology docking, molecular docking are being used for the elucidation of the roles of these transcription factors. The present review emphasizes on the different techniques used for TF identification and its function elucidation. In-silico tools form the foundation of cutting edge computational techniques which can facilitate in the development of stress resilient plants.

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Introduction:-

Heavy metals are a heterogeneous group of elements with distinct functions and chemical properties. Heavy metals are mostly categorized as transition elements in the periodic table. Heavy metals are elements with a specific weight of greater than 5 g cm3 (Bharti et al., 2022). Heavy metals are metals that have a density at least five times that of water (Tanwar et al., 2023). Heavy metals are classified as essential (Mo, Mn, Cu, Ni, Fe, Zn) or non-essential (Cd, Ni, As, Hg, Pb). Essential metals maintain human metabolism, such as Cu, which is required for hemoglobin production and carbohydrate metabolism, but excessive amounts cause cellular damage (Sharma et al., 2022). Many heavy metals are also important for plants because they act as cofactors, trigger enzyme reactions, and offer ductility, conductivity, and cation stability (Zaynab et al., 2022). However, when these metals are present in higher concentrations than required, they exhibit toxicity. Deficits in necessary heavy metals have an impact on both human health and agricultural productivity. Non-essential metals have a harmful effect even at low quantities. They do not degrade in the environment or metabolize into other intermediate molecules. Heavy metals are released into the ecosystem as a result of industrial, residential, agricultural, pharmaceutical, and technical applications, as well as natural phenomena such as volcanic explosions and rock weathering. Heavy metals are toxic, non-biodegradable, and remain in the environment for a long period. They have a negative impact on plants, humans, and animals because

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they are pollutants and thus the most significant issue. Heavy metals in ecosystems form a cyclical contamination chain that includes industry, the atmosphere, soil, water, food, and humans (Bharti et al., 2022). As mentioned in Figure 1, the heavy metals contaminate the plants and the soil in varying rates.

Heavy metals enter the human body through a variety of routes, including contaminated food, water, skin, and inhalation. These metals absorbed through the colon are primarily water soluble and go to multiple organs via the circulatory system. Heavy metals, on the other hand, impact the respiratory tract and many cells, including endothelium and epithelial cells, at low concentrations (Ali et al., 2021). Heavy metal pollution also inhibit plant growth. Metals are found in larger concentrations in soil, influencing seed germination, plant growth, production, physiological, biochemical, and genetic factors in the plant system (Ameen et al., 2022). Plant toxicity varies according to soil composition, pH, and plant characteristics.

Living things rely on the intricate process of gene expression. Gene expression is the process by which a gene's information is used to guide the synthesis of a functioning gene product. The method could perhaps explain the differences in cell types and activities among species with largely identical DNA (Mitsis et al., 2020). The complex process of gene expression entails the synchronization of several dynamic events that are regulated at several levels (Chen et al., 2021). The transcriptional, post-transcriptional, translational, and post-translational levels are among these regulatory levels. Both tissue-specific gene expression and gene activity in response to stimuli are fundamentally influenced by gene transcriptional regulation (Effhimiadou et al., 2020). Transcription factors (TFs) are the primary regulators of gene transcription. Proteins known as transcription factors (TFs) are able to bind particular DNA regions in order to regulate transcription (Chrousos et al., 2020). Different mechanisms are used by each cellular life form to initiate and regulate transcription. Eukaryotes have several specialized RNA polymerases, but prokaryotes only have one RNA polymerase (Pugh et al., 2021).TFs can be activated directly by certain molecules that bind, called ligands, or indirectly by a variety of intracellular transduction pathways (Mathelier et al., 2024)). TFs primarily control gene activity by attaching themselves to particular patterns of short DNA base pairs called motifs or cis-regulatory elements (CREs) in target genes' upstream, intron, or downstream regions.Proteins known as TFs have the ability to bind particular DNA sequences and control the expression of genes. Numerous variables, including as gene regulatory elements, chemical cofactors, and epigenetic pathways, have an impact on their evolution. The network that controls gene expression is made up of these molecular mediators and transcription factors. Clarifying the processes by which TFs have changed and how they affect the evolution of other regulatory molecules can reveal details about the evolution of organisms and the emergence of phenotypic differences (Bacopoulou et al., 2020).DNA binding domains are the particular domains that TFs possess that have the ability to bind DNA (DBD). TFs detect their target sequences using a range of DNA-binding structural motifs, such as highmobility group box (HMG), helix-turn-helix (HTH), and homeodomain (HD) (Halow et al., 2020). Transcription factors (TFs) are essential in plants because they govern gene expression, which in turn controls a variety of biological activities, including growth, development, metabolism, and stress responses. To either activate or inhibit transcription, these proteins attach to particular DNA sequences, usually in enhancer or promoter regions (Hrmova et al., 2021). Plant TFs play a crucial role in plant adaptation and survival since they are necessary for reacting to environmental stimuli such as drought, salt, temperature changes, and disease attacks. In plants, a number of transcription factor families are distinct or extremely important (Strader et al., 2022). For instance, the MYB and bZIP families control secondary metabolism and stress tolerance, whereas the AP2/ERF family is involved in hormone signaling and reactions to environmental stressors. For the development and defense mechanisms of plants, the NAC and WRKY families are very significant. The MADS-box TFs are also essential for the growth of flowers and fruits. Auxins, gibberellins, and abscisic acid are just a few of the plant hormones that many TFs interact with to regulate development and adaptability (Javed et al., 2020).

Heavy metal exposure can change gene expression and stress responses in plants by disrupting transcription factors (TFs). By dislodging necessary zinc ions and impairing their capacity to control gene expression, heavy metals including cadmium, lead, and arsenic can interfere with metal-binding TFs, especially those with zinc-finger domains (Li et al., 2022).Furthermore, in response to heavy metal stress, specific transcription factors (TFs) are activated to control detoxification and metal homeostasis genes, including Metal-Responsive Transcription Factor-1 (MTF-1) and basic helix-loop-helix (bHLH) proteins (Manzoor et al., 2022). Additionally, heavy metals produce reactive oxygen species (ROS) and oxidative stress, which can harm TFs, interfere with their ability to bind DNA, and cause epigenetic changes that further alter gene regulation. Reduced productivity and heightened vulnerability to environmental stressors can result from these disturbances, which can also affect plant growth, development, and stress tolerance (Han et al., 2022).Plants growing in soil can suffer direct harm from heavy metals, which can

negatively impact their physiological characteristics such as growth height, root development, and leaf area. Plants use systems such metal chelation, vacuolar compartmentalization, transporter modulation, and antioxidant function augmentation to combat the harmful effects of heavy metals on plant growth and development (Yu et al., 2022).



"In-silico" describes simulations or tests that are carried out using computer modeling and computational techniques as opposed to in a living creature ("in vivo") or a physical laboratory ("in vitro"). The term "in-silico analysis" describes the study of plant genes, proteins, metabolic pathways, and biological processes using bioinformatics methods and computational tools instead of physical trials. With this method, scientists may effectively study genome evolution, simulate molecular interactions, predict gene functions, and examine transcription factor binding sites (Buglak et al., 2020). In-silico techniques are frequently employed in plant research for metabolic pathway modeling, molecular docking, structural biology investigations, and genome and transcriptome analysis. These analyses aid in the comprehension of how plants react to environmental stressors such as drought, disease resistance, and heavy metal contamination. In-silico analysis speeds up research, lowers laboratory expenses, and yields insightful information that may be verified by in vitro or in vivo tests by mimicking biological processes (Chang et al., 2023).Because it is a highly precise, time-efficient, and cost-effective method of investigating plant biology, insilico analysis has several benefits for plant research. Without requiring lengthy laboratory tests, it enables researchers to assess vast amounts of genomic, proteomic, and metabolic data, allowing for the prediction of gene functions, transcription factor binding sites, and metabolic pathways. Plant samples are preserved using this nondestructive computational method, which also provides information on genetic alterations, disease resistance, and stress responses (Jiang et al., 2021). Furthermore, comparative studies between various plant species are made easier by in-silico technologies, which support crop development and evolutionary research. These analyses improve prediction accuracy and speed up plant genetics and biotechnology discoveries by combining artificial intelligence and machine learning (Samokhvalov et al., 2020). In the end, in-silico methods minimize resource consumption and laboratory waste, which supports sustainable research.

The significance of this review lies in its compilation of previous computational research on heavy metal-responsive TFs, emphasizing their regulation processes and interplay. Important TF families that are crucial for metal detoxification and homeostasis, including MYB, WRKY, bZIP, ERF, and bHLH, are also discussed (Vlachakis et al., 2020). Moreover, in-silico methods like transcriptome analysis, homology modeling, and molecular docking can forecast TF activity and aid in the creation of genetically modified plants with improved metal tolerance (Zherdev et al., 2020). This review will offer a thorough grasp of how heavy metals affect TFs by combining bioinformatics and plant molecular biology, opening the door for further studies in plant stress physiology and sustainable agriculture.

Developing crops resistant to heavy metals and enhancing global food security require such knowledge (Khan et al., 2022).

Different heavy metals studies for in-silico analysis

Arsenic (As)-

One hazardous heavy metal that has a major effect on plant development, metabolism, and productivity is arsenic (As). Because arsenate (As⁵⁺) mimics phosphate, it mainly enters plants through polluted soil and water via phosphate transporters (Balali-Mood et al., 2021). Arsenic causes oxidative stress and DNA damage in plant cells by interfering with vital physiological functions like photosynthesis, food intake, and enzyme activity. Plants use defense mechanisms such the development of antioxidant enzymes (like catalase and superoxide dismutase), metal chelation through phytochelatins and metallothioneins, and sequestration in vacuoles to stop cellular damage in order to fight arsenic toxicity (Stolarska et al., 2023). In order to reduce toxicity, arsenic (As) stress in plants sets off intricate regulatory processes governed by a number of transcription factors (TFs) that activate genes responsive to stress (Li et al., 2022). To examine these TFs, forecast their binding locations, and simulate their function in arsenic detoxification, in-silico research has been widely employed. The role of important TF families in arsenic tolerance has been investigated, including bZIP, WRKY, MYB, NAC, AP2/ERF, and Metal-Responsive Transcription Factor-1 (MTF-1) (Wagner et al., 2022). Their structural and functional characteristics have been revealed by computational methods like as homology modeling with SWISS-MODEL and Phyre2, TF binding site prediction with tools like MEME and JASPAR, and molecular docking to investigate TF-DNA interactions. Furthermore, to find TFs that are differently expressed under arsenic stress, transcriptome data mining, gene expression analysis, and protein-protein interaction studies have been carried out (Choudhury et al., 2022). The evolutionary conservation of these TFs across plant species has also been better understood thanks to comparative genomics and phylogenetic research. Designing phytoremediation techniques for arsenic-contaminated settings, creating stress-resistant plant kinds, and discovering possible genes for genetic engineering all depend on these in-silico investigations (Ghosh et al., 2022). In-silico research offer a high-throughput, cost-effective method of comprehending arsenic-responsive transcriptional regulation by fusing bioinformatics with plant stress physiology. This opens the door to more sustainable agriculture and enhanced crop resistance to heavy metal stress (Dzantiev et al., 2020).

Cadmium (Cd)-

The development, metabolism, and cellular processes of plants are all disrupted by the extremely poisonous heavy metal cadmium (Cd). It interferes with enzyme activity, water and nutrient intake, photosynthesis, and membrane damage, resulting in oxidative stress and decreased crop output(Sharif et al., 2022). It is not necessary for plant growth. Through calcium and zinc transporters, cadmium penetrates plant roots, builds up in tissues, and disrupts vital biological functions (Tanwar et al., 2023).Plants under Cd stress activate a variety of transcription factors (TFs) to control genes that respond to stress and are involved in detoxification, antioxidant defense, and metal transport. Important TF families that control metal chelation, sequestration, and oxidative stress responses—including bZIP, WRKY, MYB, NAC, AP2/ERF, and Metal Tolerance Proteins (MTPs)-are essential in reducing Cd toxicity (Khan et al., 2023). These TFs have been thoroughly examined, their DNA binding locations predicted, protein interactions modeled, and their function in Cd tolerance comprehended by in-silico investigations. Molecular docking and homology modeling (SWISS-MODEL, Phyre2) have aided in the prediction of TF-DNA interactions and structural changes under Cd stress, while computational tools including as MEME, JASPAR, and PlantPAN have been utilized to find TF binding motifs in Cd-responsive genes (Asaf et al., 2023). To find TFs that are differently expressed under Cd exposure, transcriptome and co-expression network analysis have also been carried out. These in-silico techniques offer a quick and affordable way to find Cd-responsive regulatory networks, which aids in the creation of crop varieties resistant to Cd and phytoremediation techniques for areas contaminated by Cd (Khan et al., 2023).

Lead (Pb)-

Because of its persistence and lack of biodegradability, lead (Pb) is a highly toxic heavy metal that presents significant risks to human health and the environment (Sadeghiet al., 2021). The main causes of its environmental accumulation include mining, battery waste, industrial pollutants, and tainted fertilizers. Although lead is not necessary for plants, it can enter through the roots through cation transporters, build up in plant tissues, and interfere with water balance, photosynthesis, nutrient uptake, and enzyme activity (Fatima et al., 2022). Crop productivity is eventually decreased by high Pb levels because they cause oxidative stress, damage to cell membranes, and growth suppression.Different transcription factors (TFs) that control stress-responsive genes involved in metal detoxification, transport, and antioxidant defense mechanisms are activated by plants in response to Pb poisoning (Stolarska et al., 2023). Important TF families, including bZIP, WRKY, MYB, NAC, and AP2/ERF, modulate the

expression of genes involved in Pb chelation, sequestration, and ROS scavenging, hence playing crucial roles in Pb stress tolerance (Strader et al., 2022). To study these TFs, predict their DNA binding sites, and model their function in Pb detoxification, in-silico studies have been widely employed.Pb-responsive TF binding motifs have been found using bioinformatics tools such as MEME, JASPAR, and PlantPAN, and TF-DNA interactions and structural alterations under Pb stress have been predicted with the aid of molecular docking and homology modeling (SWISS-MODEL, Phyre2) (Manzoor et al., 2022). Key TF regulatory networks implicated in Pb stress tolerance have also been identified by transcriptome research, protein-protein interaction modeling, and co-expression network analysis (Khan et al., 2023). These in-silico methods help design Pb-tolerant crop varieties and phytoremediation techniques to efficiently clear Pb-contaminated settings by offering insightful information on Pb-responsive gene regulation.

Manganese (Mn)-

An important micronutrient for plants, manganese (Mn) is involved in redox equilibrium, enzyme activation, and photosynthesis (Sharma et al., 2022). On the other hand, toxicity from excessive Mn accumulation results in oxidative stress, chlorosis, and metabolic disturbance (Paluch-Lubawa et al., 2023). Plants use a variety of transcription factors (TFs) that control genes related to Mn transport, detoxification, and stress responses to combat Mn toxicity. Through their modulation of antioxidant defense, Mn sequestration, and transporter control, key TF families such bZIP, WRKY, MYB, NAC, and AP2/ERF play important roles in maintaining Mn homeostasis (Ruas et al., 2020). To find Mn-responsive TFs, predict their binding locations, and examine their regulatory processes, a great deal of in-silico research has been done. Conserved motifs and regulatory elements in Mn-responsive genes have been identified using computational methods such as MEME, JASPAR, and PlantTFDB. TF-DNA interactions and structural changes during Mn stress have been predicted with the aid of molecular docking and homology modeling (SWISS-MODEL, Phyre2) (Xu et al., 2021). Furthermore, to identify important regulatory pathways linked to Mn tolerance, transcriptome analysis, protein-protein interaction modeling, and co-expression network investigations have been carried out. These in-silico methods help generate Mn-efficient crop varieties and enhance methods for sustainable plant growth in Mn-contaminated areas by offering insightful information on Mn stress responses (Guerra-Sá et al., 2020).

Iron (Fe)-

An important micronutrient for plants, iron (Fe) is necessary for respiration, photosynthesis, and enzyme activity (Zaynab et al., 2022). However, chlorosis, oxidative stress, and metabolic abnormalities can result from both Fe toxicity and deficiency, which can have a detrimental impact on plant growth (Tanwar et al., 2023). Plants activate particular transcription factors (TFs) that govern Fe absorption, transport, and detoxifying processes in order to maintain Fe homeostasis under stress. The bHLH (basic Helix-Loop-Helix), FIT (FER-like Iron Deficiency-Induced Transcription Factor), WRKY, MYB, and bZIP are important TF families that regulate Fe-responsive genes through chelation (phytosiderophores, nicotianamine), antioxidative defense, and Fe transporter activity (IRT1, NRAMP) (Strader et al., 2022).Numerous in-silico investigations have been carried out to examine Fe-responsive TFs and forecast their protein interactions, gene targets, and binding motifs. Conserved cis-regulatory elements in Fe-related genes have been found using bioinformatics tools such as PlantTFDB, JASPAR, and MEME. TF-DNA interactions have been predicted with the aid of molecular docking and homology modeling (SWISS-MODEL, Phyre2), and Fe stress-responsive regulatory circuits have been comprehended by transcriptome research and co-expression network analysis (Dinesh et al., 2020). By offering important insights into Fe homeostasis, these in-silico methods help create crop varieties that are efficient in iron and enhance agricultural Fe management techniques.

Important transcription factors associated with heavy metal stress WRKY-

A vast family of transcriptional regulators, including WRKY TFs, are essential to the signaling network that controls a number of plant functions. Important plant processes are influenced by the repression and activation of WRKY proteins, which normally operate as both repressors and activators (Zhang et al., 2024). According to recent research, WRKY TFs play a critical role in controlling disease resistance and plant defense. Drought and high salinity dramatically and rapidly increase the expression of numerous WRKY genes, indicating their regulatory functions in these signaling pathways. Furthermore, heavy metals can cause this expression (Lu et al., 2024). The highly conserved WRKY domain of WRKY TFs binds to W-box elements in target gene promoters to regulate a variety of biological processes, such as hormone signaling, secondary metabolite synthesis, and biotic and abiotic stress responses. By modifying stress-related gene expression, WRKY TFs improve resistance to infections, drought, salinity, heavy metal toxicity, and oxidative stress, according to studies conducted in a variety of plants, including *Arabidopsis*, rice, wheat, soybean, and maize (Li et al., 2020).

WRKY TFs in a variety of plant species have been extensively studied using in-silico research. The identification of conserved WRKY-binding motifs and cis-regulatory elements has been aided by computational tools like as PlantTFDB, JASPAR, and MEME (Pang et al., 2020). In order to categorize WRKY TFs and forecast their evolutionary relationships, phylogenetic and comparative genomic analyses have been carried out. Co-expression network analysis and transcriptome profiling have shed light on WRKY regulatory pathways under various stress situations, while molecular docking and homology modeling (SWISS-MODEL, Phyre2) have been utilized to examine WRKY-DNA interactions (Zou et al., 2021). Through genetic engineering and marker-assisted breeding, these in-silico methods help us better understand how WRKY functions and help create crops that can withstand stress.

ERF-

As a member of the AP2/ERF superfamily, the Ethylene Response Factor (ERF) transcription factor family is an important regulator of plant growth, development, and stress responses (Lu et al., 2024). Through their regulation of stress-responsive genes, ERFs are essential for mediating ethylene signaling as well as responses to pathogen assaults, heavy metal toxicity, drought, and salinity. Stress reactions associated with ethylene, a major stress hormone brought on by a variety of abiotic stimuli, are regulated by the ERF TFs. The capacity of ERF TFs to bind to stress response gene promoters led to their initial identification (Zhang et al., 2024). According to research, ERF TFs are activated by a variety of biotic and abiotic stressors, including salt, osmotic stress, drought, hypoxia, temperature variations, and pathogen infections, in addition to reacting to stress hormones including ethylene, jasmonic acid, and abscisic acid. The majority of plant ERF TFs are expressed in all tissues, but primarily in the roots, and are found in the nucleus (Zhao et al., 2022). Research conducted on a variety of plant species, including *Arabidopsis*, rice, wheat, tomato, soybean, and maize, has demonstrated that ERFs improve abiotic stress tolerance through hormone pathway modulation, antioxidant defense mechanism activation, and improved water retention. By controlling the production of secondary metabolites and pathogenesis-related (PR) genes, certain ERFs also aid in the resistance to biotic stress (Wu et al., 2022).

ERFs have been extensively studied through in-silico analysis, which includes promoter analysis, motif identification, and phylogenetic classification utilizing programs like PlantTFDB, JASPAR, and MEME. ERF-DNA interactions have been predicted using molecular docking and homology modeling (SWISS-MODEL, Phyre2), and transcriptome and co-expression network investigations provide insight into ERF-mediated regulatory pathways under stress (Xu et al., 2022). Through genetic engineering and molecular breeding, these in-silico techniques help uncover important ERFs for creating crops that can withstand stress.

MYB-

The majority of plants express the myeloblastosis oncogene (MYB) family, a sizable group of TF that is common in eukaryotes. Previous studies have demonstrated that a number of MYB TFs participate in the ABA signaling network and control how plants react to drought (Zhang et al., 2024). ABA regulates plant MYB proteins in a variety of ways, improving resistance to abiotic stress. Furthermore, MYB TFs control Cd resistance and respond to Cd stress mechanisms in plants. The majority of MYB TFs are found in the nucleus and are expressed in the plant's many tissues, primarily in the root (Lu et al., 2024). The number of MYB repetitions in the DNA-binding domain of MYB TFs determines their classification into the R1-MYB, R2R3-MYB, 3R-MYB, and 4R-MYB subfamilies. MYB TFs have been shown to play a role in secondary metabolite biosynthesis (flavonoids, anthocyanins, lignins), biotic stress defense, and abiotic stress tolerance (drought, salinity, heavy metals, oxidative stress) in a variety of plant studies, including *Arabidopsis*, rice, maize, wheat, soybean, and tomato. Auxin, gibberellins, ABA, and other hormone signaling pathways are regulated by several MYB TFs, which affect plant development and adaptability (Wang et al., 2021).

Using bioinformatics tools like PlantTFDB, JASPAR, and MEME, in-silico analysis has been widely used to explore MYB TFs, with an emphasis on phylogenetic classification, motif identification, and promoter analysis. MYB-DNA interactions and structural features have been predicted using molecular docking and homology modeling (SWISS-MODEL, Phyre2) (Zheng et al., 2021). Key MYB regulation pathways under various stress situations have been identified with the use of transcriptome and co-expression network analysis. Through genetic engineering and molecular breeding, these computational methods enable the production of stress-resistant crops by offering insightful information about MYB activities (Niu et al., 2021).

bHLH-

A vast family of plant transcription factors known as the basic helix-loop-helix (bHLH) family controls gene expression by acting as homodimers or heterodimers (Zhang et al., 2024). In plants, a number of TFs have distinct functions in controlling iron homeostasis. Despite being present in all plant tissues, subcellular localization research reveals that the majority of bHLH TFs are found in the nucleus and are primarily expressed in the root system (Lu et al., 2024). A conserved bHLH domain, which promotes DNA binding and protein dimerization, is a characteristic of bHLH TFs. By controlling stress-responsive genes, bHLH TFs have been linked to both biotic stress defense and abiotic stress tolerance (drought, salinity, iron deficiency, and heavy metals) in a variety of plant species, including *Arabidopsis*, rice, maize, wheat, tomato, and soybean (Guo et al., 2021). Furthermore, bHLH TFs are essential for hormone signaling (auxin, gibberellins, and jasmonic acid), root and shoot development, and secondary metabolism (flavonoid and anthocyanin production).

bHLH TFs have been extensively studied through in-silico analysis, which includes promoter analysis, motif identification, and phylogenetic classification using programs like PlantTFDB, JASPAR, and MEME (Asaf et al., 2023). While transcriptome and co-expression network investigations shed light on bHLH regulatory pathways, molecular docking and homology modeling (SWISS-MODEL, Phyre2) aid in the prediction of bHLH-DNA interactions. Through genetic engineering, these computational methods aid in the identification of important bHLH genes that increase agricultural productivity and stress tolerance (Khan et al., 2023).

bZIP-

A basic DNA-binding region and an adjacent leucine zipper that promotes dimerization are characteristics of basic (region) leucine zipper (bZIP) transcription factors. Numerous plants include bZIP proteins, which are involved in a range of biological activities, including light signaling, plant senescence, flower formation, and seed maturation (Lu et al., 2024). Additionally, bZIP TFs negatively regulate the growth and development of plants. According to subcellular localization research, bZIPs are expressed differently in different plant tissues but are primarily found in the nucleus of plant cells. Fewer bZIP TFs are expressed in the stems or leaves of many plants, while the majority are expressed in the roots (Chen et al., 2021).In order to activate stress-responsive pathways, bZIP TFs play a role in seed maturation, blooming, secondary metabolism, and stress adaption in a variety of plants, including *Arabidopsis*, rice, maize, wheat, soybean, and tomato. They increase plant resilience in harsh environments by controlling genes linked to hormone-mediated stress responses, osmoprotectant production, and antioxidant defense (Yao et al., 2021).

With the use of programs like PlantTFDB, JASPAR, and MEME, in-silico analysis has been extensively employed to investigate bZIP TFs through phylogenetic classification, motif finding, and promoter analysis. While co-expression network and transcriptome investigations have shed light on bZIP-regulated pathways, molecular docking and homology modeling (SWISS-MODEL, Phyre2) have assisted in the prediction of bZIP-DNA binding interactions (Jiang et al., 2021). Through the use of genetic engineering, these computational methods aid in the identification of important bZIP genes that improve agricultural performance and stress resilience.

Major in-silico approaches involved in heavy metal stress studies

Because they make it possible to identify genes, transcription factors (TFs), and regulatory networks that are responsive to stress, in-silico methods are crucial for researching how plants react to heavy metal stress. Plant genomic data can be accessed through genome and transcriptome analysis tools such as NCBI, EnsemblPlants, and Phytozome, while RNA-seq analysis tools (STAR, HISAT2, DESeq2, EdgeR) aid in the investigation of changes in gene expression under heavy metal stress (Wu et al., 2021). The identification of important TFs implicated in metal stress responses, such as WRKY, MYB, bZIP, and bHLH, is facilitated by transcription factor prediction and motif analysis tools like PlantTFDB, JASPAR, and MEME Suite. SWISS-MODEL, Phyre2, InterPro, and STRING are examples of protein structural and functional analysis tools that aid in the prediction of TF structures, interactions, and functional domains (Xu et al., 2021).

Furthermore, TF interactions with heavy metal ions are analyzed by molecular docking and dynamics simulation techniques (AutoDock, HADDOCK, GROMACS, AMBER) in order to comprehend their molecular consequences (Zherdev et al., 2020). Tools for gene ontology and pathway analysis (AgriGO, PANTHER, KEGG, and DAVID) shed light on the molecular processes and biological pathways impacted by metal toxicity. Moreover, Cytoscape and WGCNA co-expression network analysis aids in identifying important regulatory genes under stressful situations

(Buglak et al., 2020). By improving our knowledge of plant defense systems against heavy metals, these computational methods support crop improvement via molecular breeding and genetic engineering.

SI.	Transcription	Heavy	In-Silico Study	Plant Species	Effect	Citation
No.	Factor (TF)	Metal	_	_		
1	WRKY	Cd, Pb, As	TF identification, motif analysis, gene expression profiling	Rice, Arabidopsis, Wheat	Regulates metal detoxification, ROS scavenging	(Khan et al.,2023)
2	bZIP	Cd, As, Zn	Phylogenetic analysis, promoter analysis, docking studies	Maize, Tomato, Arabidopsis	Involved in stress signaling, metal chelation	(Lu et al., 2022)
3	МҮВ	Cd, Pb, Cu	Co-expression network, domain prediction, GO analysis	Rice, Maize, Barley	Enhances metal stress tolerance, activates transporters	(Wang et al., 2021)
4	bHLH	Fe, Mn, Zn	Homology modelling motif search, molecular docking	<i>Arabidopsis</i> , Soybean, Maize	Regulates iron uptake, metal homeostasis	(Yao et al., 2024)
5	ERF	Cd, Pb, Hg	Promoter binding analysis, transcriptome analysis	Tomato, Rice, Wheat	Modulates ethylene signaling in stress responses	(Khan et al., 2023)
6	NAC	As, Cd, Pb	Gene regulatory network, phylogenetic analysis	Rice, Arabidopsis, Brassica	Controls antioxidant response and metal detoxification	(Rai et al., 2022)
7	AP2/ERF	Zn, Cd, Cu	Protein-ligand interaction, docking analysis	Wheat, Tomato, Maize	Regulates root architecture and metal transporters	(Faraji et al., 2020)
8	Hsf	Pb, Cu, As	Promoter analysis, co-expression analysis	Arabidopsis, Rice, Soybean	Activates heat shock proteins for metal stress protection	(Jiang et al., 2021)
9	DREB	Cd, As, Fe	Functional domain analysis, TF binding site prediction	Maize, Rice, Barley	Regulates drought and metal stress responses	(Xia et al., 2023)
10	GRAS	Zn, Cu, Ni	Phylogenetic study, docking with metal ions	<i>Arabidopsis</i> , Wheat, Tomato	Enhances metal ion sequestration in vacuoles	(Jaiswal et al., 2022)
11	GATA	Fe, Zn, Cu	GO enrichment, gene clustering	Rice, Arabidopsis, Soybean	ControlsFehomeostasisandchlorophyllsynthesis	(Kim et al., 2021)
12	HB	Pb, Cd, Hg	Homology modelling, motif prediction	Wheat, Arabidopsis, Maize	Involved in stress signaling and root development	(Stephanou et al., 2024)
13	LBD	Zn, Pb, As	Phylogenetic and structural analysis	Arabidopsis, Rice, Maize	Modulates lateral root development in metal-stressed plants	(Huanget al., 2021)

Table 1:- Summary of transcription factors (TFs) associated with heavy metal stress in plants and their in-silico studies:

14	C2H2-ZF	Cd, Zn, Cu	Protein-ligand binding, TF-DNA	Wheat, Maize, Arabidopsis	Regulatesmetaltransportand	(Li et al., 2021)
			interaction		homeostasis	
15	ТСР	Ni, Zn,	Gene expression	Tomato, Rice,	Influences plant	(Zhou et
		Fe	correlation,	Arabidopsis	growth under heavy	al., 2022)
			pathway mapping	-	metal toxicity	
16	TGA (bZIP	Cd, Pb,	TF-protein	Wheat, Maize,	Enhances oxidative	(Duan et
	subfamily)	Zn	interaction, docking	Rice	stress response and	al., 2024)
	• •		analysis		metal detoxification	
17	HSF	Cu, Cd,	Structural	Arabidopsis,	Increases stress	(Hong et
		Fe	modeling, promoter	Rice, Soybean	resilience by	al., 2022)
			enrichment analysis		activating chaperone	
					proteins	
18	SPL	Cd, Zn,	Co-expression and	Maize, Tomato,	Modulates metal	(Li et al.,
		Pb	regulatory network	Maize	transporters and root	2024)
			analysis		architecture	
19	HMG	Fe, Zn,	Molecular docking,	Arabidopsis,	Regulates chromatin	(Villano et
		As	TF-DNA	Rice, Maize	remodeling in metal	al., 2020)
			interaction		stress	
20	Trihelix (GT	Cd, Cu,	Gene enrichment,	Arabidopsis,	Enhances metal ion	(Hu et al.,
	factors)	Pb	promoter analysis	Maize, Rice	binding and	2023)
					sequestration	

 Table 2:- Summary of different in-silico tools in heavy metal stress studies.

Sl. No.	In-Silico Tools Utility in Heavy Metal Stress Studies		Citation	
1	PlantTFDB	Identification and classification of transcription factors	(Liu et al., 2024)	
2	MEME Suite	Motif discovery and promoter element analysis	(Tariq et al., 2022)	
3	JASPAR	Prediction of TF binding sites in promoter regions	(Rauluseviciute et al., 2024)	
4	STRING	Protein-protein interaction (PPI) network analysis	(Gable et al., 2021)	
5	SWISS-MODEL	Homology modeling of transcription factor structures	(Huang et al., 2024)	
6	AutoDock	Molecular docking for TF-DNA and TF-metal interactions	(Badshah et al., 2020)	
7	Cytoscape	Visualization and analysis of gene co-expression networks	(Majeed et al., 2023)	
8	KEGG	Pathway enrichment analysis of metal stress- responsive genes	(Kanehisa et al., 2023)	
9	WGCNA	Weighted gene co-expression network analysis for TF studies	(Chen et al., 2024)	
10	DAVID	Gene ontology (GO) enrichment analysis	(Kuo et al., 2024)	
11	InterPro	Functional domain prediction in TFs	(Blum et al., 2021)	
12	Pfam	Identification of conserved protein domains in TFs	(Yang et al., 2022)	
13	PlantRegMap	Regulatory network analysis of plant transcription factors	(Tian et al., 2020)	
14	PANTHER	Functional classification of TF families	(Ebert et al., 2021)	
15	HADDOCK	Protein-ligand docking for studying TF-metal	(Saponaro et al.,	

		interactions	2020)
16	Phyre2	Protein structure prediction and homologymodeling	(Hayati et al., 2021)
17	AgriGO	GO analysis specific to plant species	(Varunjikar et al., 2023)
18	PlantCARE	Identification of cis-regulatory elements in TF promoters	(Fennimoreet al., 2020)
19	PLACE	Prediction of stress-responsive elements in promoter regions	(Ilyas et al., 2021)
20	iTOL	Phylogenetic tree visualization for TF classification	(Rizk et al., 2021)

Conclusion:-

The investigation of heavy metal stress-responsive transcription factors (TFs) in plants has been transformed by insilico analysis, which makes it possible to explore gene regulatory mechanisms in large quantities at a reasonable cost. PlantTFDB, MEME Suite, JASPAR, STRING, AutoDock, and other bioinformatics tools make it easier to identify, categorize, and functionally characterize TFs implicated in metal stress adaption (Zherdev et al., 2020). Using methods like gene ontology (GO) enrichment, protein-protein interaction (PPI) networks, motifidentification, promoter analysis, and molecular docking, scientists can forecast the functions of important TFs in reducing heavy metal toxicity (Jiang et al., 2021).

Through the combination of transcriptomics, genomes, and structural bioinformatics, in-silico studies offer important new information about the regulatory networks controlling how plants react to heavy metal stress (Shabbir et al., 2020). This information is crucial for creating crops that can withstand stress through genetic engineering and molecular breeding. Additionally, by addressing worldwide issues with soil contamination and crop yield, these computational methods support sustainable agriculture and phytoremediation techniques (Afzal et al., 2020). The precision and relevance of in-silico research in plant stress biology will be significantly improved by upcoming developments in AI-driven modeling, machine learning techniques, and multi-omics data integration.

Future Prospects:-

By combining cutting-edge computational and experimental methods, in-silico investigation of heavy metal stressresponsive transcription factors (TFs) in plants will have a bright future. A better understanding of TF-mediated stress responses will be possible through the integration of multi-omics technologies, including proteomics, metabolomics, transcriptomics, and genomes (Samokhvalov et al., 2020). Key stress-responsive components will be more precisely identified as a result of improved TF prediction, regulatory network modeling, and functional annotation brought about by the application of machine learning (ML) and artificial intelligence (AI). Molecular docking and dynamics simulations will also help uncover new resistance mechanisms by providing better insights into TF-metal interactions (Wu et al., 2021). The development of metal-tolerant agricultural varieties can be accelerated through the use of CRISPR-based genome editing backed by in-silico predictions. Furthermore, growing databases of plant-specific stress responses will guarantee improved data accessibility and uniformity for upcoming studies. The production of genetically modified plants with improved metal tolerance, environmental cleanup, and sustainable agriculture will all benefit from in-silico methods as computational tools advance (Shahzad et al., 2022).

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