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# Comparative Computational Analysis of Key Drought-Responsive Proteins Across Plant Species: Insights into Molecular Adaptations for Stress Tolerance

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## Abstract

**Background:** Drought stress is a major abiotic factor limiting plant productivity, necessitating adaptive mechanisms at the molecular level to ensure survival. **Methods:** This study presents a comparative computational analysis of the physicochemical properties of five drought-responsive proteins (bZIP1, AP2-EREBP, COX1, HSP20 and PKDP) across multiple plant species, including *Oryza sativa*, *Zea mays*, *Triticum aestivum*, *Sorghum bicolor*, *Arabidopsis thaliana* and *Hordeum vulgare*. The amino acid composition, molecular weight (MW), isoelectric point (pI), and instability index were analyzed using the ProtParam tool to elucidate the structural adaptations of these proteins under drought conditions. **Results:** The results indicate that bZIP1 and AP2-EREBP exhibit serine and glycine content variations, respectively, suggesting distinct phosphorylation-mediated regulatory mechanisms for drought adaptation. COX1, a mitochondrial enzyme, displays high leucine and glycine levels, reinforcing its conserved role in energy metabolism during drought-induced oxidative stress. HSP20 is characterized by high valine content and a complete absence of cysteine, enhancing its chaperone activity and structural flexibility for protein stabilization under osmotic stress. PKDP, a kinase involved in drought-responsive signaling, exhibits species-specific differences in lysine content and instability index, indicating variations in phosphorylation-dependent regulatory functions. **Conclusions:** Overall, this comparative study highlights key biochemical adaptations that enhance protein stability, flexibility, and function under drought stress. These findings provide molecular insights into plant drought resilience and lay the groundwork for targeted genetic improvement strategies in crop breeding programs. Further experimental validation, including structural modeling and functional assays, is recommended to confirm the computational predictions and explore their potential applications in enhancing plant stress tolerance.

**Keywords:** Drought stress, heat shock proteins, cytochrome c oxidase, protein kinases, computational analysis.

## 1. Introduction

Drought stress is a major abiotic factor limiting plant growth, development, and productivity worldwide. Plants have evolved complex regulatory mechanisms to mitigate the adverse effects of drought, including physiological adjustments, metabolic reprogramming, and molecular responses such as transcriptional regulation, protein stabilization and signal transduction [1,2]. Among these, specific proteins play crucial roles in enhancing drought tolerance by modulating stress-responsive pathways at the cellular and molecular levels.

Several key protein families have been identified as central players in drought stress adaptation, including bZIP1, AP2-EREBP, COX1, HSP20, and PKDP. Each of these proteins contributes uniquely to drought tolerance mechanisms in plants. bZIP1 (Basic Leucine Zipper 1) transcription factors regulate drought-induced gene expression, particularly by modulating abscisic acid (ABA)-dependent signaling pathways [3,4]. Similarly, AP2-EREBP (APETALA2/Ethylene-Responsive Element Binding Protein) transcription factors enhance drought resis

tance by mediating stress-signaling cascades and regulating dehydration-responsive element-binding (DREB) proteins [5].

Mitochondrial function also plays a vital role in drought adaptation, as energy homeostasis is critical for sustaining stress responses. COX1 (Cytochrome c Oxidase Subunit 1), a key enzyme in the mitochondrial electron transport chain, is crucial for ATP production under drought conditions. Its structural stability and flexibility are essential for maintaining energy metabolism in plants experiencing water deficits [6,7].

Chaperone proteins such as HSP20 (Heat Shock Protein 20) are indispensable for protecting cellular proteins from drought-induced denaturation. HSP20 functions as a molecular chaperone, preventing aggregation and refolding misfolded proteins, thus contributing to cellular homeostasis during water scarcity [8,9].

Finally, protein kinases serve as central regulators of drought stress signaling. PKDP (Protein Kinase Drought-Responsive Protein) is involved in phosphorylation-dependent regulation of drought-responsive pathways, influencing signal transduction and post-translational modifications that optimize plant responses to water limitations [10].

Despite the significance of these proteins in drought stress adaptation, their structural and functional properties across different plant species remain underexplored. Comparative analysis of their amino acid composition, molecular weight, isoelectric point (pI) and instability index can provide insights into their evolutionary conservation and functional adaptations to drought stress.

This study employs computational analysis using the ProtParam tool to characterize the physicochemical properties of bZIP1, AP2-EREBP, COX1, HSP20, and PKDP across multiple plant species, including *Oryza sativa*, *Zea mays*, *Triticum aestivum*, *Sorghum bicolor*, *Arabidopsis thaliana,* and *Hordeum vulgare*. By examining interspecific variations, this research aims to elucidate the structural adaptations that optimize these proteins for drought stress resilience. The findings will contribute to a deeper understanding of plant stress physiology and provide a basis for genetic improvement strategies targeting crop drought tolerance.

## 2. Materials and Methods

### Protein Sequence Selection and Retrieval

The protein sequences of bZIP1, AP2-EREBP, COX1, HSP20, and PKDP were retrieved from publicly available databases, including UniProt and NCBI, for multiple plant species, including *Oryza sativa*, *Zea mays*, *Triticum aestivum*, *Sorghum bicolor*, *Arabidopsis thaliana* and *Hordeum vulgare*. These proteins were selected based on their established roles in drought stress response, including transcriptional regulation (bZIP1, AP2-EREBP), mitochondrial energy metabolism (COX1), molecular chaperone activity (HSP20) and signal transduction (PKDP). The protein name, organism, and number of amino acids are presented in **Table 1**.

### Computational Analysis of Protein Properties

The physicochemical properties of the selected proteins were analyzed using ProtParam, a tool available on the ExPASy server according to [11].

**This analysis provided key biochemical parameters, including:**

**Amino acid composition**: The relative abundance of amino acids was examined to infer structural and functional adaptations to drought stress.

**Molecular weight (MW)**: The MW of each protein was calculated to understand variations in protein size and its implications for cellular functions.

**Isoelectric point (pI)**: The theoretical pI was determined to assess protein charge properties under physiological conditions, influencing protein-protein and protein-DNA interactions.

**Instability index (II)**: A predictive measure of protein stability was calculated, with values above 40 indicating potential instability and rapid turnover in vivo as reported by [12].

### Comparative Analysis Across Plant Species

The biochemical properties of each protein were compared across plant species to identify patterns of conservation and divergence.

### Data Interpretation and Statistical Validation

The results were analyzed to determine the functional implications of the observed physicochemical differences. Comparative analysis was performed using statistical tools available in ExPASy as outlined by [11]. Literature-based validation was conducted to correlate computational findings with experimentally validated drought stress mechanisms in plants using the method described by [10,13].

**Table (1)** Numbers of Amino Acid of five key drought-responsive proteins in different plants

| **Organism** | **No. of AA** | | | | |
|---|---|---|---|---|---|
| | **AP2-EREBP** | **bZIP 1** | **COX1** | **Hsp 20** | **PKDP** |
| **Oryza Sativa** | 250 | 436 | 524 | 150 | 785 |
| **Zizania palustris** | 250 | 446 | 524 | 150 | 764 |
| **Zea mays** | 258 | 405 | 528 | 152 | 755 |
| **Sorghum bicolor** | 265 | 410 | 530 | 151 | 783 |
| **Triticum aestivum** | 258 | 388 | 524 | 151 | 754 |
| **Arabidopsis thaliana** | 242 | 411 | 527 | 155 | 719 |
| **Hordeum vulgare** | 250 | 396 | 526 | 151 | 755 |

## 3. Results and discussion

### The Role of AP2-EREBP Transcription Factor in Drought Stress Adaptation: A Comparative Analysis Across Plant Species

The AP2-EREBP (APETALA2/Ethylene-Responsive Element Binding Protein) transcription factor family plays a pivotal role in plant responses to abiotic stresses, including drought stress. These transcription factors regulate gene expression in response to environmental cues, contributing to stress adaptation mechanisms such as osmotic balance, stomatal regulation, and antioxidant activity [**5**]. The present study utilizes ProtParam analysis to examine the amino acid composition and physicochemical properties of AP2-EREBP across multiple plant species, linking these characteristics to drought tolerance strategies.

### Amino Acid Composition and Its Implications for Drought Stress Tolerance

Amino acid composition is a key determinant of protein function, influencing stability, flexibility, and interaction dynamics. The analysis of AP2-EREBP across different plant species reveals significant variations in amino acid content, which may reflect species-specific adaptations to drought conditions. Amino Acid (%) of AP2-EREBP in different plants are shown in **Table 2** and **Figure 1**.

**Alanine (A):** Alanine plays a role in maintaining protein stability and energy production under stress conditions, making it crucial for plants experiencing prolonged drought [14]. Alanine content ranges from 7.9 % in *Arabidopsis thaliana* to 13.6% in *Oryza sativa*.

**Glycine (G):** Glycine contributes to protein flexibility and stress adaptation, particularly in proteins involved in DNA-binding and transcriptional regulation [15]. The glycine content varies between 5.4 % (*Arabidopsis thaliana*) and 8.4 % (*Hordeum vulgare*).

**Serine (S):** Serine plays a critical role in phosphorylation events, a key post-translational modification regulating drought-responsive transcriptional activity [16]. High serine levels may enhance regulatory efficiency, particularly under drought conditions requiring rapid signal transduction. The highest serine content is found in *Triticum aestivum (14.0%)*, with the lowest in *Oryza sativa* (11.6%).

Differences in glycine and alanine content indicate structural flexibility and stress-adaptive modifications in monocots versus dicots [16]. These compositional differences suggest that monocot species, such as *Oryza sativa* and *Triticum aestivum*, may have evolved a more stable AP2-EREBP variant that enhances transcriptional control during drought stress, whereas *Arabidopsis thaliana* relies on rapid phosphorylation-dependent regulatory mechanisms.

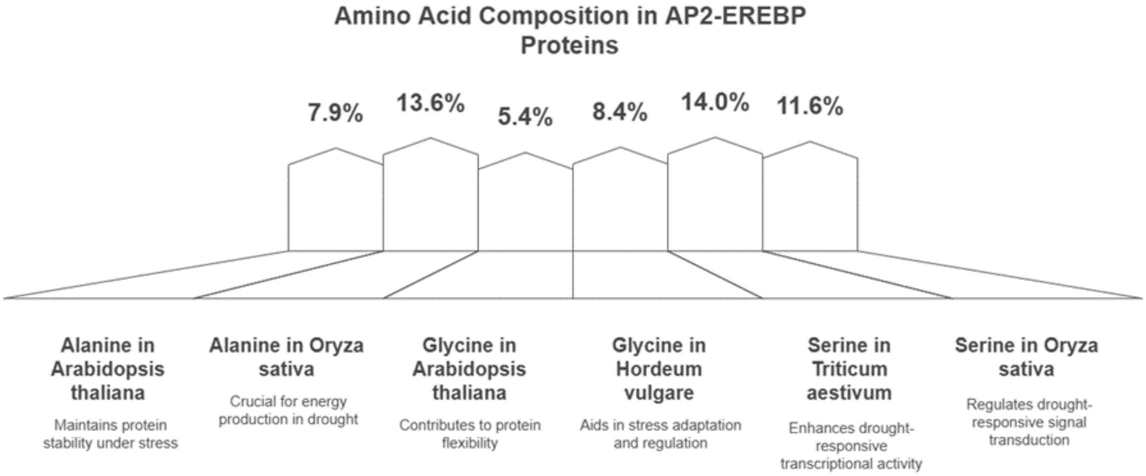

**Fig. (1)** Amino Acid Composition in Plant Species and Their Role in Drought Stress Tolerance

Figure (1), illustrates the relative composition of key amino acids in various plant species and their functional roles in drought stress adaptation. Alanine in *Arabidopsis thaliana* (7.9%) contributes to protein stability under stress, while in *Oryza sativa* (13.6%), it plays a crucial role in energy production during drought conditions. Glycine, present in *Arabidopsis thaliana* (5.4%) and *Hordeum vulgare* (8.4%), is associated with protein flexibility and stress adaptation, respectively. Serine, with notable abundance in *Triticum aestivum* (14.0%) and *Oryza sativa (11.6%).* plays critical roles in transcriptional activity and phosphorylation, which are essential for drought response mechanisms. The figure highlights the significance of amino acid composition in plant species and their biochemical contributions to stress tolerance.

### Physicochemical Properties and Their Relationship to Drought Stress Adaptation

Several physicochemical characteristics of AP2-EREBP influence its stability and functionality in stress responses:

**Molecular Weight (MW):** The MW of AP2-EREBP is relatively conserved across species, ranging from 26686.60Da (*Hordeum vulgare*) to 28348.24Da (*Sorghum bicolor*). This small variation suggests that while minor structural differences exist, they do not significantly impact the transcription factor's role in drought stress adaptation.

**Isoelectric Point (pI):** The pI values range from 4.91 (*Sorghum bicolor*) to 6.04 (*Arabidopsis thaliana*). A lower pI is associated with increased acidity, which can enhance protein-DNA and protein-protein interactions under stress conditions [17]. The acidic nature of *Arabidopsis thaliana* AP2-EREBP may facilitate efficient transcriptional regulation by improving DNA-binding efficiency, particularly in stress-responsive pathways.

**Instability Index (II):** The instability indices for AP2-EREBP variants exceed 40 in all species, classifying them as unstable proteins [12]. The highest instability index (70.35) is observed in *Triticum aestivum*, indicating a potential requirement for stabilizing protein-protein interactions or post-translational modifications to maintain functional activity. This instability may be an adaptive feature, allowing for rapid degradation and turnover, ensuring timely regulatory responses to fluctuating drought conditions. Molecular Weight, Isoelectric Point and Instability Index of AP2-EREBP are shown in, **Table 3** and **Figure 2.**

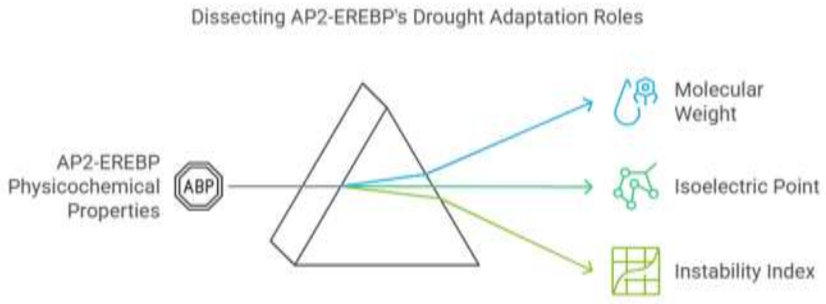

**Fig. (2)** Dissecting AP2-EREBP’s Drought Adaptation Roles

Figure (2), illustrates the physicochemical properties of AP2-EREBP (APETALA2/Ethylene-Responsive Element Binding Proteins) and their potential contributions to drought stress adaptation. The diagram highlights three key properties: molecular weight (blue), which influences protein stability and interactions; isoelectric point (green), which affects charge distribution and binding affinity under varying pH conditions; and instability index (light green), which provides insights into the protein’s turnover rate and structural resilience under drought conditions. These parameters collectively contribute to AP2-EREBP's role in stress response and plant adaptation mechanisms.

**Functional and Evolutionary Adaptations to Drought Stress**

The observed differences in AP2-EREBP properties highlight key evolutionary adaptations that optimize drought stress tolerance:

**Monocot vs. Dicot Adaptations:** The divergence in amino acid composition and physicochemical traits between *Arabidopsis thaliana* (dicot) and monocot species suggests distinct drought response strategies. Monocots tend to have higher alanine and glycine content, which may contribute to enhanced structural stability and transcriptional efficiency under prolonged drought stress.

**Post-Translational Regulation in *Arabidopsis thaliana*:** The higher serine content in *Arabidopsis thaliana* supports a phosphorylation-dependent regulatory mechanism, allowing for rapid activation and deactivation of stress-responsive genes. This feature is particularly advantageous in environments where drought conditions fluctuate, requiring quick adaptive responses [16].

**Compensatory Mechanisms for Protein Stability:** Despite being classified as unstable, AP2-EREBP likely utilizes stabilizing interactions with co-factors or undergoes regulatory modifications such as phosphorylation and ubiquitination to maintain functional integrity during drought stress as obtained by [18].

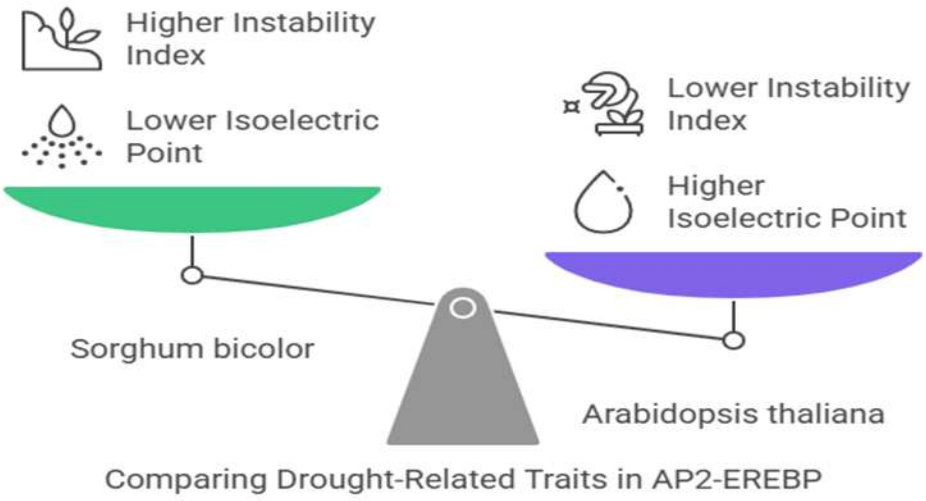

**Fig. (3)** Comparative Analysis of Drought-Related Traits in AP2-EREBP Transcription Factors of *Sorghum bicolor* and *Arabidopsis thaliana*

Figure (3) presents a comparative evaluation of the physicochemical properties of AP2-EREBP transcription factors in *Sorghum bicolor* and *Arabidopsis thaliana*, which play a critical role in drought stress response. *Sorghum bicolor* exhibits a higher instability index and lower isoelectric point (pI), indicating potential susceptibility to rapid degradation and a more acidic protein environment. Conversely, *Arabidopsis thaliana* demonstrates a lower instability index and higher isoelectric point, suggesting greater protein stability and a more basic pH environment. These properties influence protein function, regulatory efficiency, and overall plant resilience under drought conditions. The balance between stability and biochemical characteristics provides insights into species-specific drought adaptation strategies.

## The Role of bZIP1 in Drought Stress Tolerance: A Comparative Analysis Across Plant Species

The bZIP1 transcription factor plays a crucial role in regulating gene expression in response to environmental stressors, particularly drought stress. Understanding its biochemical properties across different plant species can provide valuable insights into its stability, functional efficiency and evolutionary adaptations [3]. This study examines the physicochemical properties of bZIP1 using ProtParam analysis, with a specific focus on its implications for drought stress tolerance.

### Amino Acid Composition and Drought Stress Response

Amino acid composition significantly influences protein structure and function, particularly in response to abiotic stress. Amino Acid (%) of bZIP1 in different plants are shown in, **Table 2** and **Figure 4**.

**Several residues identified in bZIP1 are directly linked to drought stress resilience:**

**Proline (P):** High proline content, especially in *Triticum aestivum* (10.3%), is noteworthy. Proline plays a well-established role in osmoprotection, stabilizing proteins, and maintaining cellular homeostasis under drought conditions [19]. Plants with higher proline content in bZIP1 may exhibit greater drought resistance by enhancing protein stability and reducing oxidative damage.

**Serine (S) and Threonine (T):** These amino acids are essential for phosphorylation, a key regulatory mechanism in stress signaling pathways [20]. *Arabidopsis thaliana*, which possesses the highest serine content (13.4%), is known for its rapid adaptation to drought stress through ABA-dependent signaling pathways. **Glycine (G):** Increased glycine levels in *Triticum aestivum* (9.0%) and *Sorghum bicolor* (8.1%) may enhance protein flexibility, facilitating dynamic interactions required for drought-induced gene regulation. Variations in serine and proline content suggest adaptations in phosphorylation-dependent stress responses and protein stability under drought conditions [20,4]. The observed variations suggest that monocot species, particularly cereals like *Triticum aestivum*, may have evolved a bZIP1 variant that confers enhanced structural resilience under drought conditions, whereas dicots such as *Arabidopsis thaliana* prioritize phosphorylation-based rapid response mechanisms.

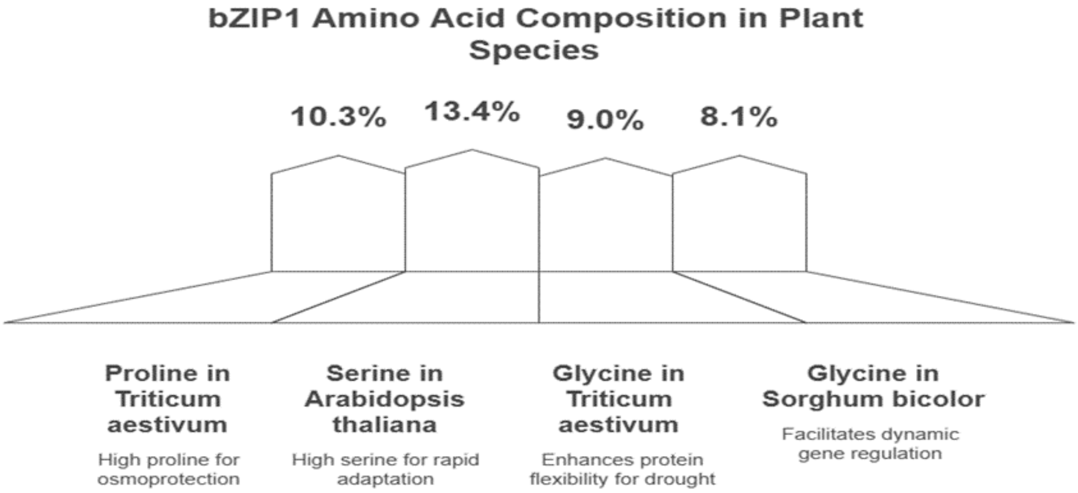

**Fig. (4)** Comparative Amino Acid Composition of bZIP1 Transcription Factor in Different Plant Species

Figure (4), illustrates the amino acid composition of the bZIP1 transcription factor across four plant species: *Triticum aestivum*, *Arabidopsis thaliana*, and *Sorghum bicolor*, highlighting key amino acids associated with drought stress tolerance. Proline (10.3%) in *T. aestivum* plays a significant role in osmoprotection under drought conditions. Serine (13.4%) in *A. thaliana* is essential for rapid adaptation to environmental stress. Glycine (9.0%) in *T. aestivum* enhances protein flexibility, contributing to improved drought resilience. Additionally, glycine (8.1%) in *S. bicolor* facilitates dynamic gene regulation, which is crucial for stress adaptation. These findings underscore the role of bZIP1 in mediating plant responses to water deficit conditions through species-specific amino acid composition.

### Physicochemical Properties and Their Implications for Drought Resistance

Several physicochemical characteristics of bZIP1 influence its stability, interaction potential and regulatory efficiency in drought stress conditions. Molecular Weight, Isoelectric Point and Instability Index of bZIP 1 are showed in, **Table 3** and **Figure 5**.

**Molecular Weight (MW):** The MW of bZIP1 varies from 41351.37Da (*Triticum aestivum*) to 47738.35Da (*Zizania palustris*). Higher MW proteins often have increased functional complexity [21], potentially allowing *Zizania palustris* to engage in a broader range of drought-related regulatory interactions

**Isoelectric Point (pI):** The pI values range from 4.84 (*Oryza sativa subsp. japonica*) to 6.33 (*Triticum aestivum*). Lower pI values indicate a more acidic nature, which can enhance DNA-binding efficiency under cellular stress conditions [22]. The acidic nature of *Oryza sativa* bZIP1 may improve its transcriptional regulation during drought stress by facilitating interactions with negatively charged DNA.

**Instability Index:** All analyzed variants of bZIP1 are classified as unstable proteins, with instability indices exceeding 40. The highest instability index (63.11) in *Hordeum vulgare* suggests that its bZIP1 protein has a shorter half-life, potentially requiring chaperone assistance for functional stability under drought stress. Despite this classification, bZIP1 stability may be enhanced through post-translational modifications such as phosphorylation, which have been observed in other transcription factors regulating drought responses.

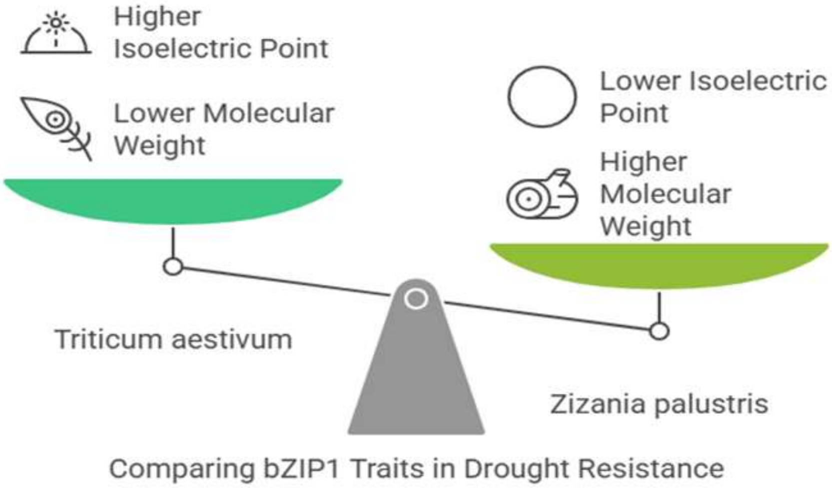

**Figure (5).** Schematic comparison of bZIP1 transcription factors from *Triticum aestivum* (left) and *Zizania palustris* (right), highlighting differences in molecular weight and isoelectric point that may underlie variations in drought tolerance. The *T. aestivum* bZIP1 is depicted with a higher isoelectric point and lower molecular weight, whereas the *Z. palustris* bZIP1 exhibits a lower isoelectric point and higher molecular weight. These contrasting physicochemical properties could reflect distinct adaptive strategies for coping with water-deficit stress in each species.

**Functional and Evolutionary Adaptations to Drought Stress**

The comparative analysis of bZIP1 across plant species reveals critical evolutionary adaptations:

**Monocot vs. Dicot Adaptations:** The divergence in amino acid composition and physicochemical properties between *Arabidopsis thaliana* (dicot) and cereals (monocots) suggests distinct drought adaptation strategies. Monocots exhibit higher proline content and greater structural stability, whereas dicots emphasize rapid regulatory phosphorylation events.

**Enhanced Phosphorylation Potential in *Arabidopsis thaliana*:** The high serine and threonine content in *Arabidopsis thaliana* may contribute to its ability to quickly activate drought-responsive genes, making it more efficient in rapid stress responses compared to cereals that rely on structural stability.

**Compensatory Mechanisms for Protein Stability:** Although bZIP1 is inherently unstable, functional compensatory mechanisms such as protein-protein interactions and post-translational modifications likely mitigate degradation. These adaptations are crucial for sustaining bZIP1 function under prolonged drought stress.

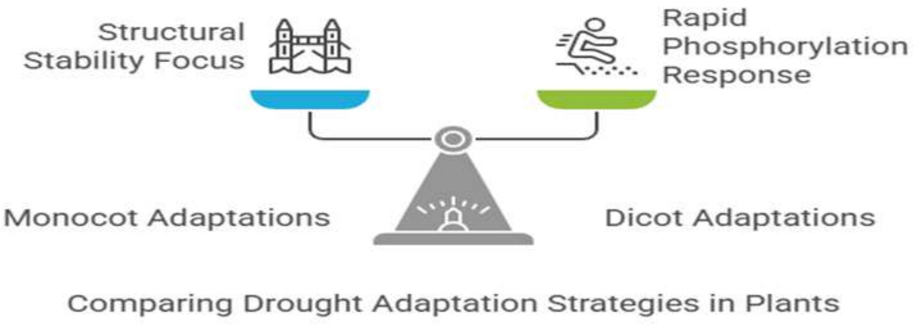

**Fig.(6)**. Schematic depiction comparing two primary drought adaptation strategies in plants. Monocots (blue scale pan) predominantly focus on structural stability, employing robust morphological traits to withstand water deficit. In contrast, dicots (green scale pan) rely on rapid phosphorylation signaling to quickly adjust physiological processes under stress. The balance emphasizes the complementary yet distinct pathways by which these plant groups mitigate drought conditions.

### The Role of COX1 in Drought Stress Adaptation: A Comparative Analysis Across Plant Species

Cytochrome c oxidase subunit 1 (COX1) is a vital component of the mitochondrial electron transport chain, responsible for catalyzing the final step of oxidative phosphorylation. Its role in cellular respiration directly impacts energy production, which is crucial for maintaining physiological functions under drought stress [6]. The ability of plants to adapt to drought conditions is often linked to mitochondrial efficiency, as oxidative phosphorylation provides the energy required for stress response mechanisms, including osmotic adjustment and reactive oxygen species (ROS) detoxification [11]. In this study, we analyze the amino acid composition and physicochemical properties of COX1 across multiple plant species to assess its role in drought stress adaptation.

### Amino Acid Composition and Its Implications for Drought Stress Tolerance

The amino acid composition of COX1 is highly conserved across different plant species, indicating its functional importance in maintaining mitochondrial integrity during stress conditions. Amino Acid (%) of COX1 in different plants are shown in, **Table 2** and **Figure 7**.

**Leucine (L):** Leucine is the most abundant amino acid in COX1, with the highest percentage observed in *Arabidopsis thaliana* (11.6%). Leucine plays a key role in hydrophobic interactions and protein folding, essential for the structural stability of membrane-bound proteins like COX1 [17]. Stability under drought stress conditions is critical as mitochondrial proteins must remain functional despite dehydration and oxidative damage.

**Glycine (G):** Glycine content is consistently high across all species, with *Arabidopsis thaliana* and *Sorghum bicolor* exhibiting the highest level (10.4%). Glycine contributes to protein flexibility, which is crucial for the conformational changes required during electron transport [7]. This flexibility allows COX1 to maintain electron flow under fluctuating environmental conditions, ensuring efficient ATP production even during drought-induced metabolic shifts.

**Phenylalanine (F)**: Present in significant amounts across all species, with *Oryza sativa subsp. japonica,* containing the highest percentage (9.4%). Phenylalanine contributes to hydrophobic interactions that stabilize the protein's tertiary structure [17]. This stability is particularly important under drought stress, as proteins must maintain their functional conformation despite osmotic stress-induced cellular dehydration.

High leucine and glycine content across species highlights the conserved role of COX1 in mitochondrial electron transport and energy metabolism during drought stress [6,7]. The conserved amino acid composition of COX1 across species suggests that structural stability and flexibility are essential traits for mitochondrial function under drought conditions.

These characteristics enable plants to sustain ATP production while mitigating stress-induced mitochondrial dysfunction.

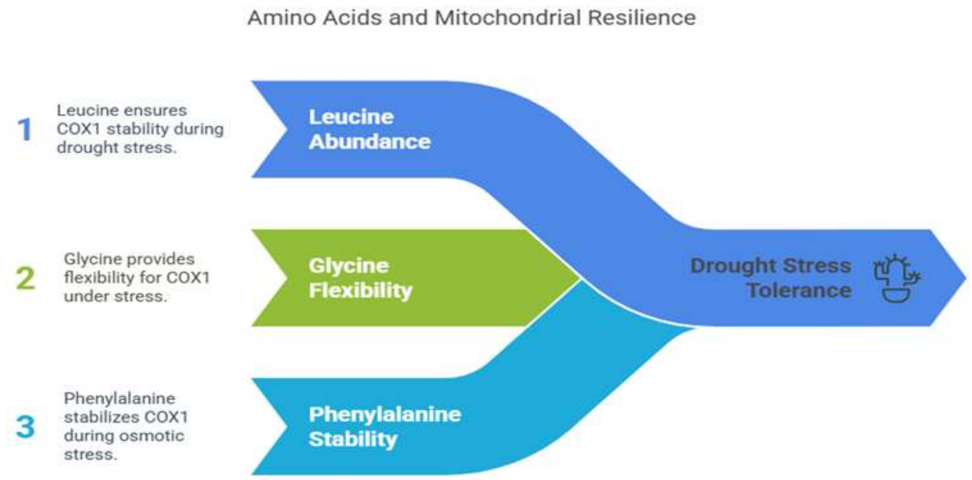

**Fig. (7).** Schematic overview illustrating how three key amino acids (leucine, glycine, and phenylalanine) contribute to mitochondrial resilience and drought stress tolerance. Leucine supports COX1 stability under water-deficit conditions, glycine confers structural flexibility that enables COX1 to function efficiently during stress, and phenylalanine fortifies COX1 under osmotic challenges. Together, these amino acid-mediated mechanisms enhance overall plant tolerance to drought.

### Physicochemical Properties and Their Relationship to Drought Stress Adaptation

The physicochemical properties of COX1 influence its stability and interaction potential, impacting its efficiency in drought-stressed conditions. Molecular Weight, Isoelectric Point and Instability Index of COX1 are showed in, **Table 3** and **Figure 8**.

**Molecular Weight (MW):** COX1 MW ranges from 57738.93Da in Zizania palustris to 58522.81Da in Sorghum bicolor. This variation may be due to differences in protein length or post-translational modifications that affect COX1 function. Larger COX1 proteins may incorporate additional regulatory sites or structural adaptations that enhance resilience under drought conditions.

**Isoelectric Point (pI):** The pI values range from 7.79 (*Arabidopsis thaliana*) to 8.41 (*Oryza sativa subsp. japonica*), indicating that COX1 proteins are generally basic. This property suggests that COX1 maintains a net positive charge at physiological pH, which may enhance interactions with negatively charged mitochondrial components, stabilizing the electron transport process under stress. A higher pI may also facilitate electrostatic interactions that improve protein stability in response to drought-induced ionic imbalances.

**Instability Index:** All COX1 variants are classified as unstable, with instability indices ranging from 29.81 (*Arabidopsis thaliana*) to 32.89 (*Zea mays*). While instability suggests a shorter half-life, it may also indicate an adaptive feature that allows for rapid protein turnover and dynamic regulatory control in response to environmental changes. Plants experiencing drought stress often modulate mitochondrial protein expression levels, and COX1 instability may allow for efficient degradation and replacement under fluctuating conditions.

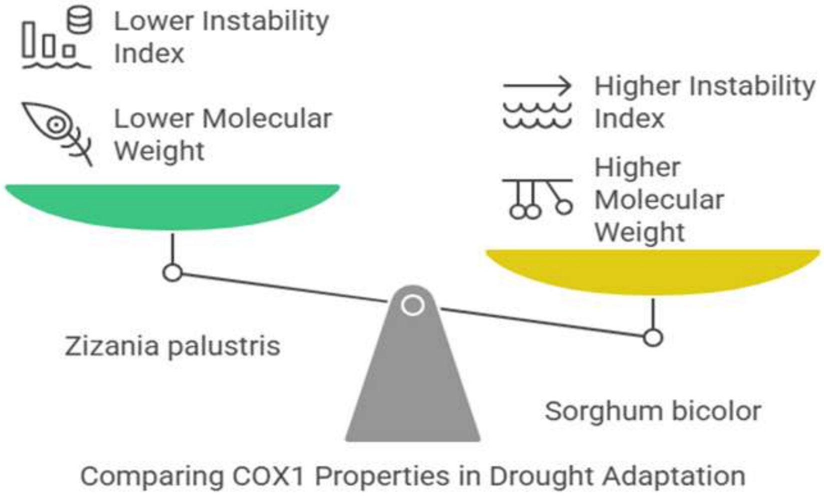

**Fig. (8).** Schematic comparison of COX1 properties in *Zizania palustris* (left) and *Sorghum bicolor* (right), highlighting differences in molecular weight and protein instability indices. *Z. palustris* exhibits a lower molecular weight and lower instability index, whereas *S. bicolor* displays a higher molecular weight and higher instability index. These contrasting physicochemical attributes may influence each species' capacity for drought adaptation through differential COX1 stability and functionality under water-limited conditions.

### Functional and Evolutionary Adaptations to Drought Stress

The highly conserved nature of COX1 across species highlights its evolutionary importance in maintaining cellular respiration under stress conditions. Several key adaptations related to drought stress tolerance can be inferred:

**Mitochondrial Stability and Drought Resilience:**

The high leucine and glycine content in COX1 enhances its stability and flexibility, essential traits for maintaining electron transport efficiency under drought stress as reported by [6]. These properties help prevent mitochondrial dysfunction and energy deficits that can occur during prolonged water scarcity.

**Electrostatic Adaptations for Stress Resistance**:

The generally basic nature of COX1, reflected in its pI values, suggests an evolutionary adaptation that enhances its interaction with other mitochondrial proteins. This characteristic may facilitate the formation of protective protein complexes that mitigate oxidative damage and sustain ATP production under drought conditions.

**Protein Turnover and Stress Adaptation:**

The instability of COX1 may be a strategic adaptation that allows for rapid degradation and synthesis in response to environmental stress. Plants undergoing drought stress often exhibit changes in mitochondrial proteome composition, and the ability to regulate COX1 expression dynamically may provide an advantage in optimizing energy metabolism during fluctuating water availability.

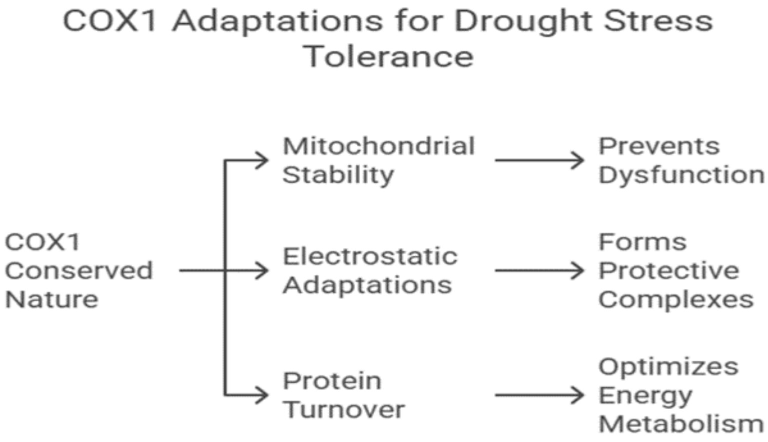

**Fig. (9).** Conceptual diagram illustrating how the conserved nature of COX1 underlies three key adaptations for drought stress tolerance. Mitochondrial stability (top branch) prevents dysfunction under water-limited conditions, electrostatic adaptations (middle branch) enable the formation of protective complexes, and protein turnover (bottom branch) helps optimize energy metabolism. Collectively, these mechanisms enhance plant resilience by maintaining COX1 functionality during drought.

### The Role of HSP20 in Drought Stress Adaptation: A Comparative Analysis Across Plant Species

Heat shock proteins (HSPs) are essential molecular chaperones that protect cellular proteins from stress-induced denaturation. Among them, the small heat shock protein (sHSP) family, particularly HSP20, plays a crucial role in plant responses to abiotic stresses, including drought stress [8]. HSP20 functions by preventing protein aggregation and facilitating refolding, ensuring cellular homeostasis under unfavorable conditions [9]. This study analyzes the amino acid composition and physicochemical properties of HSP20 across different plant species, linking these features to its role in drought stress adaptation.

### Amino Acid Composition and Its Implications for Drought Tolerance

The amino acid composition of HSP20 varies across plant species but maintains conserved features that contribute to its functional role in drought stress resistance. Amino Acid (%) of Hsp 20 in different plants are showed in, **Table 2** and **Figure 10**.

**Valine (V):** Valine, a hydrophobic amino acid, stabilizes protein structures through hydrophobic interactions, which are essential for maintaining protein integrity under drought-induced dehydration stress [17]. High valine content is observed in *Oryza sativa subsp. japonica* (13.3%) and *Triticum aestivum* (13.2%). The abundance of valine suggests that HSP20 enhances structural stability, preventing protein denaturation during osmotic stress.

**Glutamic Acid (E):** Glutamic acid enhances protein solubility and charge balance, enabling HSP20 to interact efficiently with other biomolecules under drought stress conditions [7]. This characteristic may help stabilize proteins that are prone to aggregation under dehydration stress. *Sorghum bicolor* and *Triticum aestivum* show elevated glutamic acid levels (11.9%).

**Cysteine (C):** Notably absent in all analyzed species (0.0%), indicating a lack of disulfide bonds. The absence of cysteine suggests that HSP20 relies on non-covalent interactions for its function, which may contribute to its conformational flexibility, allowing rapid adaptation to changing cellular environments during drought stress.

**Lysine (K):** Lysine’s positive charge enhances electrostatic interactions, which may facilitate HSP20’s association with nucleic acids and other negatively charged molecules involved in drought stress signaling pathways [23]. *Arabidopsis thaliana* (11.0%) and *Hordeum vulgare* (10.6%) exhibit high lysine content. The increased lysine content in *Arabidopsis thaliana* suggests a potential regulatory role in phosphorylation-mediated signaling under drought conditions.

The absence of cysteine and high valine levels across species suggests functional flexibility in chaperone activity, facilitating protein stability under osmotic stress [24,9]. These amino acid characteristics indicate that HSP20 enhances protein stability and solubility under drought stress, preventing aggregation and maintaining cellular homeostasis.

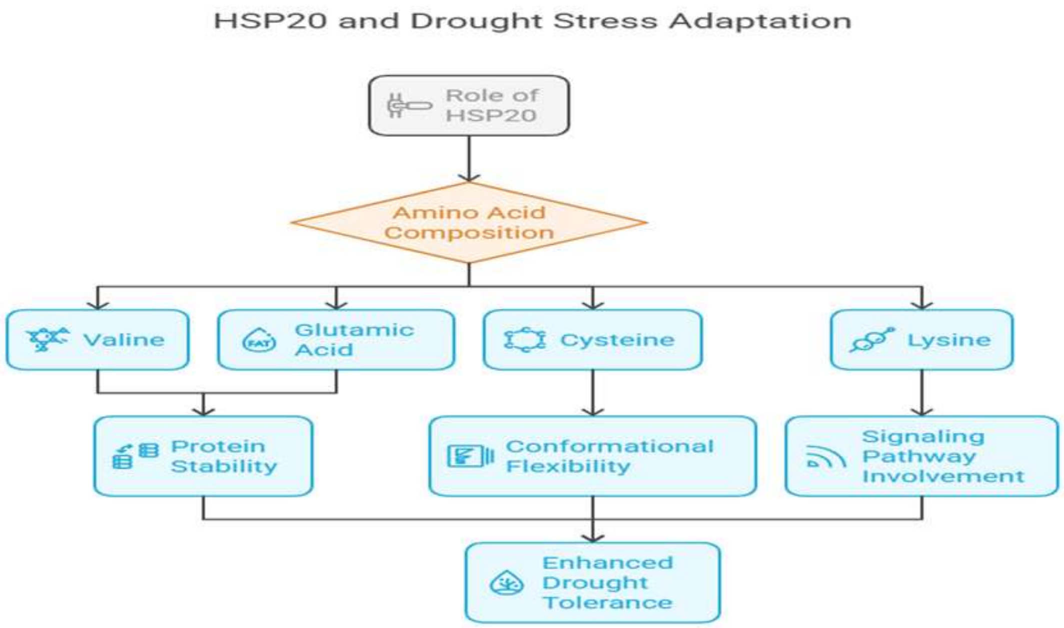

**Fig. (10)** The Role of HSP20 in Drought Stress Adaptation via Amino Acid Composition

Figure (10), presents a schematic representation of the role of HSP20 in drought stress adaptation, emphasizing the contribution of specific amino acids to drought tolerance mechanisms. The amino acid composition of HSP20 includes valine, glutamic acid, cysteine, and lysine, each playing a distinct role in stress adaptation. Valine and glutamic acid contribute to protein stability, ensuring proper protein folding and structural integrity under drought conditions. Cysteine enhances conformational flexibility, allowing proteins to maintain functional dynamics despite environmental stress. Lysine plays a critical role in signaling pathway involvement, facilitating cellular communication, and stress response regulation. Collectively, these amino acids contribute to enhanced drought tolerance, highlighting the functional significance of HSP20 in plant stress adaptation.

### Physicochemical Properties and Their Relationship to Drought Stress Adaptation

Several physicochemical traits of HSP20 impact its function and stability in drought-stressed environments. Molecular Weight, Isoelectric Point and Instability Index of Hsp 20 are showed in, **Table 3** and **Figure 11**.

**Molecular Weight (MW):** The MW of HSP20 ranges from 16895.15Da in *Hordeum vulgare* to 17,623.94Da in *Arabidopsis thaliana*. The variation in MW may reflect species-specific differences in post-translational modifications or sequence length, which can influence stress-responsive properties [11]. Larger molecular weights may indicate additional phosphorylation sites that enhance regulatory functions under drought conditions.

**Isoelectric Point (pI):** The pI values range from 5.56 (*Sorghum bicolor*) to 6.85 (*Arabidopsis thaliana*), suggesting an acidic to near-neutral charge. A lower pI indicates a preference for acidic environments, such as the cytosol or lysosomes, where stress-induced protein aggregation is mitigated [25]. This feature may allow HSP20 to function effectively in cellular compartments that accumulate misfolded proteins during drought stress.

**Instability Index:**
All analyzed HSP20 variants are classified as unstable (instability index >40), with the highest instability in *Sorghum bicolor* (61.84). High instability suggests rapid protein turnover, a characteristic of regulatory proteins involved in transient stress responses [12]. This may enable plants to synthesize HSP20 quickly in response to drought stress and degrade it when no longer needed, conserving cellular resources.

The physicochemical properties of HSP20 suggest that its stability, solubility, and rapid turnover are key features that contribute to its effectiveness in drought stress adaptation.

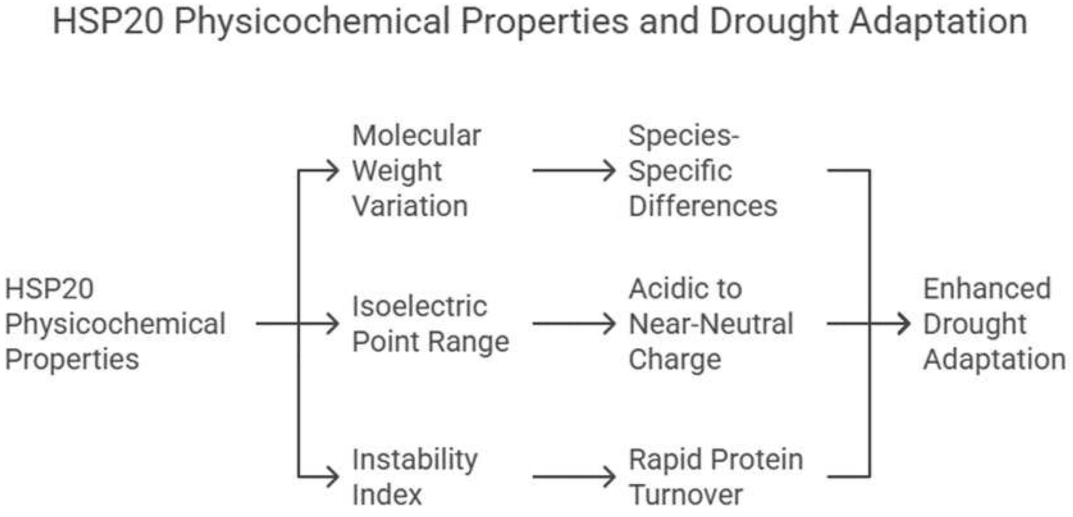

**Fig. (11)** HSP20 Physicochemical Properties and Their Role in Drought Adaptation.

Figure(11), illustrates the relationship between the physicochemical properties of HSP20 and drought adaptation mechanisms in plants. Molecular weight variation leads to species-specific differences, influencing the functional diversity of HSP20 proteins. The isoelectric point range, spanning from acidic to near-neutral charge, affects protein solubility and interactions under drought stress conditions. The instability index, indicative of protein turnover rates, facilitates rapid protein turnover, enabling dynamic responses to stress. These physicochemical attributes collectively contribute to enhanced drought adaptation, highlighting the role of HSP20 in maintaining cellular homeostasis under drought conditions.

**Functional and Evolutionary Adaptations to Drought Stress**
The conserved absence of cysteine and the high valine content across species suggest that HSP20 is optimized for flexibility and transient interactions, which are essential for its role as a molecular chaperone during drought stress. Several key adaptations highlight its function in drought tolerance:

**Structural Flexibility for Stress Adaptation:**
The absence of cysteine enhances HSP20's conformational plasticity, allowing it to interact with multiple client proteins under drought stress conditions. This flexibility ensures that HSP20 can respond dynamically to fluctuating environmental conditions, preventing protein misfolding and aggregation.

**Hydrophobic Interactions for Protein Stabilization:**
The high valine content suggests that hydrophobic interactions play a critical role in HSP20's ability to stabilize denatured proteins under drought stress [17]. This trait enables plants to maintain cellular function even during severe dehydration.

**Electrostatic Interactions in Stress Signaling:**
The elevated lysine content in *Arabidopsis thaliana* indicates a potential role in

phosphorylation-dependent signaling pathways. Phosphorylation enhances HSP20's regulatory capacity, facilitating its activation and interaction with stress-related proteins during drought conditions [23].

**Rapid Turnover for Dynamic Regulation:** The high instability index suggests that HSP20 is rapidly synthesized and degraded, ensuring a swift and adaptable response to environmental stressors[12]. This feature is advantageous in drought-prone environments where quick activation and deactivation of stress-responsive proteins are essential for survival.

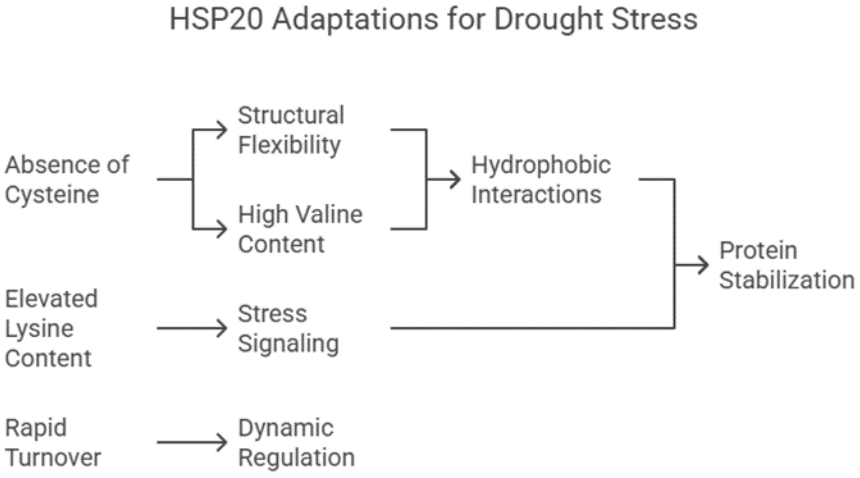

**Fig. (12)** HSP20 Adaptations for Drought Stress.

Figure (12), illustrates the key molecular adaptations of HSP20 that contribute to drought stress resilience in plants. The absence of cysteine enhances structural flexibility, while high valine content promotes hydrophobic interactions, collectively leading to protein stabilization under drought conditions. Elevated lysine content facilitates stress signaling, enabling effective cellular responses to dehydration stress. Additionally, rapid protein turnover supports dynamic regulation, ensuring a swift adaptive mechanism to environmental fluctuations. These molecular features highlight the functional importance of HSP20 in drought tolerance.

## The Role of PKDP in Drought Stress Adaptation: A Comparative Analysis Across Plant Species

Protein kinases play an essential role in plant signal transduction pathways, particularly in response to environmental stressors such as drought. The Protein Kinase Drought-Responsive Protein (PKDP) is a key regulatory protein that influences stress adaptation mechanisms, including osmotic balance, reactive oxygen species (ROS) detoxification, and cellular signaling pathways [10]. This study examines the amino acid composition and physicochemical properties of PKDP across various plant species, linking these features to its potential role in drought stress adaptation.

## Amino Acid Composition and Its Implications for Drought Tolerance

The amino acid composition of PKDP varies among plant species, indicating potential differences in function and stability under drought conditions. The Amino Acid (%) of PKPD in different plants are shown in **Table 2** and **Figure 13**.

**Alanine (A):** Alanine is known for its role in protein stability and metabolic regulation during drought stress [26]. The highest alanine content is observed in *Oryza sativa subsp. japonica* (13.2%) and *Oryza sativa subsp. indica* (13.1%), whereas *Arabidopsis thaliana* has significantly lower alanine levels (5.6%). The high alanine content in rice suggests that PKDP may contribute to drought resilience by maintaining structural integrity and metabolic efficiency.

**Glycine (G):** Glycine enhances protein flexibility and stability, which are critical for maintaining functional conformation under drought-induced osmotic stress [26]. Glycine levels are consistently high across species, ranging from 9.2% in *Arabidopsis thaliana* to 12.1% in *Zea mays* and *Sorghum bicolor*.

**Leucine (L):** Leucine plays a key role in protein-protein interactions and cellular energy homeostasis under stress conditions [13]. An essential hydrophobic amino acid, leucine is highly represented in all species, with the highest proportion in *Arabidopsis thaliana* (13.4%).

**Lysine (K):** *Arabidopsis thaliana* exhibits the highest lysine content (5.7%), which may enhance PKDP's interactions with negatively charged molecules, including nucleic acids and phospholipids involved in drought response signaling pathways.

Variability in lysine content and instability indices across species suggest differences in phosphorylation-mediated signaling and protein turnover in drought stress adaptation [27]. These variations in amino acid composition suggest that monocots such as *Oryza sativa* and *Zea mays* prioritize alanine and glycine for structural stability, while *Arabidopsis thaliana* exhibits higher lysine content, potentially enhancing regulatory interactions during drought stress.

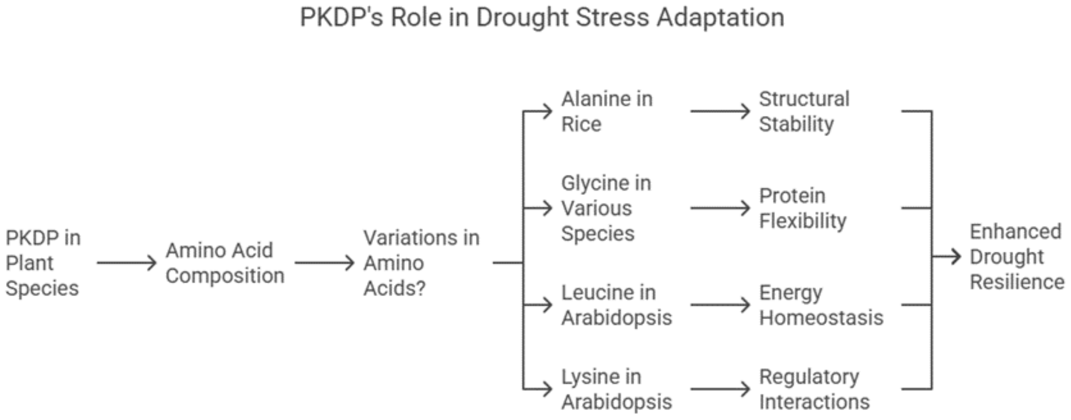

**Fig. (13)** PKDP's Role in Drought Stress Adaptation.

Figure (13), illustrates the role of PKDP in drought stress adaptation across different plant species, highlighting the impact of amino acid composition on drought resilience. Alanine in rice contributes to structural stability, while glycine in various species enhances protein flexibility, aiding in cellular protection under dehydration stress. In Arabidopsis, leucine plays a critical role in energy homeostasis, ensuring metabolic balance, whereas lysine facilitates regulatory interactions that modulate stress response pathways. Together, these amino acid variations contribute to enhanced drought resilience, emphasizing the functional significance of PKDP in plant adaptation to water scarcity.

## Physicochemical Properties and Their Relationship to Drought Stress Adaptation

The physicochemical characteristics of PKDP influence its stability and interaction potential, which are essential for effective function in drought-stressed conditions. Molecular Weight, Isoelectric Point and Instability Index of PKDP are showed in, **Table 3** and **Figure 14.**

**Molecular Weight (MW):** PKDP's molecular weight varies across species, with the highest MW observed in *Oryza sativa subsp. japonica* (81,384.41Da) and the lowest in *Arabidopsis thaliana* (77,171.58Da). Larger molecular weights may indicate additional phosphorylation sites or structural adaptations that enhance PKDP's regulatory functions under drought conditions [13].

**Isoelectric Point (pI):** The pI values range from 6.16 (*Arabidopsis thaliana*) to 9.00 (*Zizania palustris*). A lower pI suggests increased acidity, which may enhance electrostatic interactions with other stress-responsive proteins and facilitate signal transduction during drought stress as obtained by [27]. The higher pI in *Zizania palustris* suggests that PKDP in monocots may function more efficiently in neutral to basic environments.

**Instability Index:** The instability index, which predicts protein stability in a cellular environment, suggests that PKDP is generally unstable across all species. The highest instability index is observed in *Hordeum vulgare* (43.70), while the lowest is in *Arabidopsis thaliana* (36.31). This instability may indicate a short intracellular half-life, requiring post-translational modifications or protein complex formation to maintain functional stability.

The variability in these physicochemical properties suggests that PKDP is fine-tuned for species-specific drought adaptation, with dicots such as *Arabidopsis thaliana* prioritizing acidic interactions for rapid signaling, while monocots may rely on structural stability and post-translational modifications.

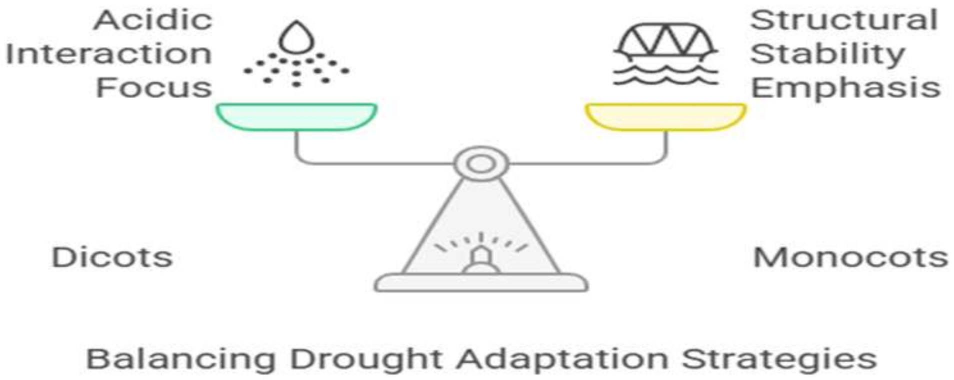

**Fig. (14)** Balancing Drought Adaptation Strategies in Dicots and Monocots.

Figure (14), represents the contrasting drought adaptation strategies between dicots and monocots. Dicots emphasize acidic interaction focus, potentially enhancing protein solubility and molecular interactions under stress conditions. In contrast, monocots prioritize structural stability, reinforcing cellular integrity and resilience against dehydration. The balance scale metaphor illustrates how these divergent approaches contribute to overall drought adaptation, reflecting evolutionary trade-offs in plant species facing water scarcity.

### Functional and Evolutionary Adaptations to Drought Stress

Several key functional adaptations of PKDP across species highlight its role in drought stress tolerance:

**Regulatory Function in Stress Signaling:** The higher lysine content in *Arabidopsis thaliana* suggests that PKDP is more involved in phosphorylation-dependent regulatory networks that respond rapidly to drought conditions.

**Structural Stability in Monocots:** The high alanine and glycine content in monocots such as *Oryza sativa* and *Zea mays* may enhance PKDP’s ability to withstand protein denaturation and maintain function under prolonged drought stress.

**Dynamic Turnover and Post-Translational Regulation:** The high instability index across species suggests that PKDP undergoes rapid degradation and turnover, ensuring precise and timely regulation of stress responses. This feature aligns with the need for dynamic control of protein kinase activity during environmental fluctuations as obtained by [10].

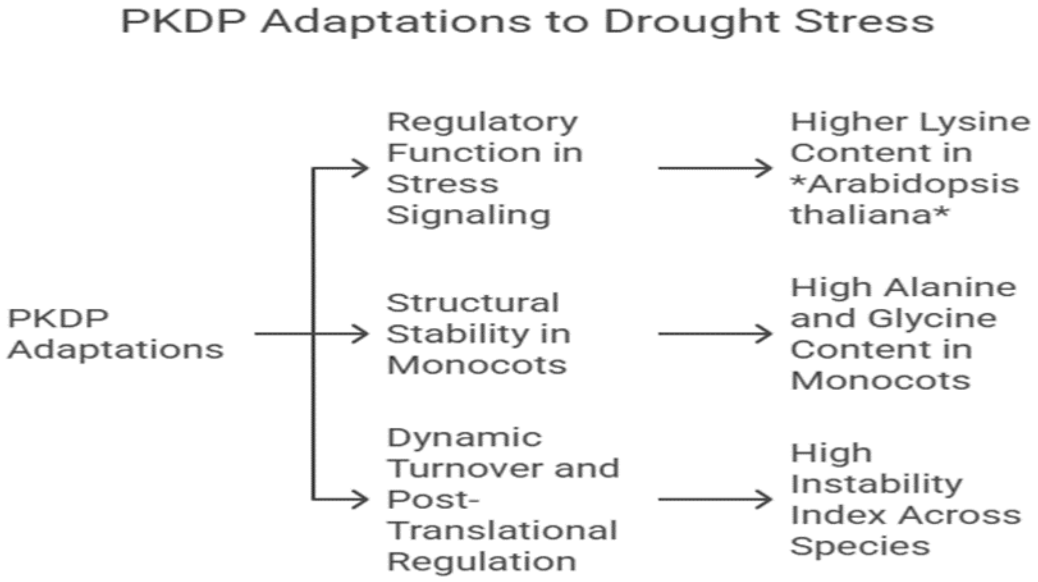

**Fig. (15)** PKDP Adaptations to Drought Stress.

Figure (15), illustrates the key adaptive roles of PKDP (Plant Kinase Domain-Containing Proteins) in response to drought stress across different plant species. The regulatory function in stress signaling is associated with higher lysine content in *Arabidopsis thaliana*, suggesting a role in protein-protein interactions and signaling efficiency. In monocots, structural stability is maintained through elevated alanine and glycine content, contributing to protein rigidity and resilience under dehydration. Additionally, PKDPs exhibit dynamic turnover and post-translational regulation, reflected in a high instability index across species, potentially facilitating rapid adaptation to fluctuating environmental conditions.

**Table (2)** Percentage of Amino Acid (%) of five key drought-responsive proteins in different plants.

| **A.A** / **Organism** | **AP2-EREBP** | | | **bZIB 1** | | | | **COX1** | | | **Hsp 20** | | | | **PKDP** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **A** | **G** | **S** | **P** | **S** | **T** | **G** | **L** | **G** | **F** | **V** | **E** | **C** | **K** | **A** | **G** | **L** | **K** |
| **Oryza Sativa** | 13.6 | 7.6 | 11.6 | 7.1 | 10.1 | 1.8 | 7.1 | 11.3 | 10.3 | 9.4 | 13.3 | 10.0 | 0.0 | 10.0 | 13.2 | 11.1 | 11.7 | 2.5 |
| **Zizania palustris** | 11.2 | 6.0 | 12.0 | 8.7 | 11.0 | 4.9 | 7.0 | 11.3 | 10.3 | 9.4 | 13.3 | 10.0 | 0.0 | 10.0 | 11.9 | 10.5 | 11.5 | 3.1 |
| **Zea mays** | 10.9 | 8.1 | 12.0 | 7.4 | 12.6 | 2.7 | 7.9 | 11.2 | 10.2 | 9.3 | 11.2 | 10.5 | 0.0 | 9.2 | 11.5 | 12.1 | 12.8 | 3.0 |
| **Sorghum bicolor** | 12.1 | 7.9 | 12.1 | 7.9 | 11.1 | 3.0 | 8.1 | 11.3 | 10.4 | 9.2 | 10.6 | 11.9 | 0.0 | 9.3 | 12.5 | 12.1 | 11.9 | 2.7 |
| **Triticum aestivum** | 12.0 | 7.8 | 14.0 | 10.3 | 12.6 | 6.7 | 9.0 | 11.1 | 10.3 | 9.4 | 13.2 | 11.9 | 0.0 | 9.9 | 11.3 | 10.1 | 12.1 | 3.3 |
| **Arabidopsis thaliana** | 7.9 | 5.4 | 13.6 | 7.3 | 13.4 | 6.3 | 4.9 | 11.6 | 10.4 | 9.3 | 9.7 | 9.7 | 0.0 | 11.0 | 5.6 | 9.2 | 13.4 | 5.7 |
| **Hordeum vulgare** | 11.2 | 8.4 | 12.8 | 6.8 | 11.6 | 3.3 | 7.8 | 11.4 | 10.3 | 9.3 | 13.2 | 11.9 | 0.0 | 10.6 | 11.0 | 10.1 | 11.9 | 3.2 |

**A**=Alanine, **G**= Glycine, **S**=Serine, **P**=Proline, **T**=Threonine, **L**=Leucine, **F**= Phenylalanine, **V**=Valine, **E**=Glutamic Acid, **C**=Cysteine, **K**=Lysine

**Table (3)** Physic-chemical properties of five key drought-responsive proteins in different plants.

| **Phy-Ch** / **Organism** | **AP2-EREBP** | | | **bZIP 1** | | | **COX1** | | | **Hsp 20** | | | **PKDP** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **M.W** | **P.I** | **I.I** | **M.W** | **P.I** | **I.I** | **M.W** | **P.I** | **I.I** | **M.W** | **P.I** | **I.I** | **M.W** | **P.I** | **I.I** |
| **Oryza Sativa)** | 26.80 | 5.12 | 64.04 | 46.03 | 4.84 | 57.89 | 57.9 | 8.41 | 31.51 | 16.19 | 6.18 | 52.24 | 81.4 | 8.65 | 38.66 |
| **Zizania palustris** | 27.34 | 5.29 | 63.65 | 47.3 | 4.97 | 59.79 | 57.9 | 8.41 | 31.51 | 16.19 | 6.18 | 52.24 | 80.1 | 9.00 | 38.85 |
| **Zea mays** | 27.72 | 5.19 | 66.51 | 42.9 | 5.22 | 58.24 | 58.4 | 8.35 | 32.89 | 17.4 | 5.81 | 50.9 | 78.5 | 8.66 | 39.70 |
| **Sorghum bicolor** | 28.24 | 4.91 | 67.09 | 42.06 | 5.34 | 55.16 | 58.8 | 8.41 | 31.24 | 17.2 | 5.56 | 61.84 | 81.8 | 8.34 | 38.38 |
| **Triticum aestivum** | 27.50 | 5.82 | 70.35 | 41.3 | 6.33 | 61.77 | 57.9 | 7.87 | 30.24 | 16.19 | 5.83 | 48.63 | 79.4 | 8.48 | 42.34 |
| **Arabidopsis thaliana** | 26.66 | 6.04 | 47.41 | 45.05 | 5.29 | 60.35 | 57.3 | 7.79 | 29.81 | 17.9 | 6.85 | 50.24 | 77.5 | 6.16 | 36.31 |
| **Hordeum vulgare** | 26.60 | 5.50 | 66.11 | 41.3 | 5.20 | 63.11 | 57.2 | 8.41 | 30.76 | 16.15 | 5.83 | 49.02 | 79.7 | 8.60 | 43.70 |

**MW**: Molecular Weight kDa, **PI**: Isoelectric Point, **II**: Instability Index

## 5. Conclusion

Drought stress imposes significant constraints on plant growth and productivity, necessitating the activation of molecular mechanisms that enhance resilience. This study conducted a comparative computational analysis of five key drought-responsive proteins (bZIP1, AP2-EREBP, COX1, HSP20 and PKDP) across multiple plant species to identify their structural and functional adaptations. By analyzing their amino acid composition, molecular weight (MW), isoelectric point (pI) and instability index using ProtParam, we gained insights into their role in drought stress adaptation.

The findings indicate that bZIP1 and AP2-EREBP exhibit variations in serine, glycine, and alanine content, which likely contribute to phosphorylation-dependent regulatory mechanisms involved in drought response. These transcription factors play a crucial role in activating stress-related genes through ABA-dependent and ABA-independent pathways [3,4]. COX1, a core mitochondrial enzyme, maintains high leucine and glycine content, reinforcing its essential role in energy metabolism and ROS detoxification during drought-induced oxidative stress [6,7].

HSP20, a molecular chaperone, is characterized by high valine content and a complete absence of cysteine, indicating its role in protein stabilization and refolding under osmotic stress conditions [8,9]. The structural flexibility of HSP20 allows it to mitigate protein misfolding, a critical function under water-deficient conditions. PKDP, a kinase involved in drought-responsive signaling, exhibits species-specific variations in lysine content and instability index, suggesting that phosphorylation-dependent regulation is optimized differently across plant taxa**[**10].

Despite the inherent instability of these proteins, their functions are likely maintained through post-translational modifications and interactions with co-regulatory proteins. The distinct adaptations observed across monocot and dicot species highlight the evolutionary divergence in drought resistance strategies, with monocots prioritizing structural stability (e.g., alanine and glycine enrichment) and dicots emphasizing rapid regulatory signaling (e.g., higher lysine content for phosphorylation).

## Future Directions

The insights gained from this computational study provide a foundation for future experimental research. Structural modeling, site-directed mutagenesis and functional assays should be conducted to validate these findings and further explore the role of these proteins in drought stress adaptation. Additionally, genetic engineering and selective breeding programs could leverage these molecular insights to enhance crop resilience to drought stress, ensuring sustainable agricultural productivity in water-limited environments.

## References

[1] Bartels, D. and Sunkar, R. (2005). "Drought and salt tolerance in plants." *Critical Reviews in Plant Sciences, 24*(1), 23-58.

[2] Nakashima, K., Takasaki, H., Mizoi, J., & Shinozaki, K. (2012). "NAC transcription factors in plant abiotic stress responses." Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 1819(2), 97-103.

[3] Jakoby, M., et al. (2002). "bZIP transcription factors in Arabidopsis." Trends in Plant Science, 7(3), 106-111.

[4] Liu, J. and Howell, S. H. (2010). "bZIP transcription factors in plant stress responses." Annual Review of Plant Biology, 61, 563-583.

[5] Franco-Zorrilla, J. M., López-Vidriero, I., Carrasco, J. L., Godoy, M., Vera, P., and Solano, R. (2014). "DNA-binding specificities of plant transcription factors and their potential to define target genes." Proceedings of the National Academy of Sciences, 111(6), 2367-2372.

[6] Kadenbach, B., Jarausch, J., Hartmann, R. and Merle, P. (1983). "Separation of mammalian cytochrome c oxidase into 13 polypeptides by a sodium dodecyl sulfate-gel electrophoretic procedure." Analytical Biochemistry, 129(2), 517-521.

[7] Creighton, T. E. (1993). Proteins: Structures and Molecular Properties (2nd ed.). W.H. Freeman.

[8] Vierling, E. (1991). "The roles of heat shock proteins in plants." Annual Review of Plant Physiology and Plant Molecular Biology, 42(1), 579-620.

[9] Kampinga, H. H., Hageman, J., Vos, M. J., Kubota, H., Tanguay, R. M., Bruford, E. A. and Craig, E. A. (2009). "Guidelines for the nomenclature of the human heat shock proteins." Cell Stress and Chaperones, 14(1), 105–111.

[10] Kosová, K., Vítámvás, P., Prášil, I. T., and Renaut, J. (2011). "Plant proteome changes under abiotic stress—contribution of proteomics studies to understanding plant stress response." Journal of Proteomics, 74(8), 1301-1322.

[11] Wilkins, M. R., Gasteiger, E., Bairoch, A., Sanchez, J. C., Williams, K. L., Appel, R. D. and Hochstrasser, D. F. (1999). "Protein identification and analysis tools in the ExPASy server." Methods in Molecular Biology, 112, 531-552.

[12] Guruprasad, K., Reddy, B. V. and Pandit, M. W. (1990). "Correlation between stability of a protein and its dipeptide composition: A novel approach for predicting in vivo stability of a protein from its primary sequence." Protein Engineering, 4(2), 155–161.

[13] Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., and Bourne, P. E. (2000). "The Protein Data Bank." Nucleic Acids Research, 28(1), 235-242.

[14] Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., and James, E. 2000.In: Molecular Cell Biology. Tenney, S., Ahr, K., Steyn, R., and Ueno, K., Eds., W.H. Freeman and Co., New York.

[15] Petsko GA, Ringe D (2004) Protein structure and function. New Science Press Ramachandran GN, Ramakrishnan C, Sasisekharan V (1963) Stereochemistry of polypeptide chain configurations. J Mol Biol 7:95–99

[16] Mann, M. and Jensen, O. N. (2003). "Proteomic analysis of post-translational modifications." Nature Biotechnology, 21(3), 255–261.

[17] Kyte, J. and Doolittle, R. F. (1982). "A simple method for displaying the hydropathic character of a protein." Journal of Molecular Biology, 157(1), 105-132.

[18] Vázquez-Hernández, M.C. , Parola-Contreras, I. , Montoya-Gómez, L.M. , Torres-Pacheco, I. , Schwarz, D. and Guevara-González, R.G. 2019. Eustressors: Chemical and physical stress factors used to enhance vegetables production. Sci. Hortic. (Amsterdam)., 250: 223–229. Vera-Reyes, I. , Vázquez-Núñez, E. , Lira-Saldivar, R.H. and Méndez-Argüello, B. 2018. Effects of nanoparticles on germination, growth, and plant crop development. Agric. Nanobiotechnology.

[19] Paul E. Verslues1 and Sandeep Sharma. (2010). Proline Metabolism and Its Implications for Plant-Environment Interaction. American Society of Plant Biologists, The Arabidopsis Book, 1- 23.

[20] Komatsu, S., & Hossain, Z. (2013). "Organ-specific proteome analysis for identification of abiotic stress response mechanism in crop." Frontiers in Plant Science, 4, 228.

[21] Shunichi Kosugia,b,c, Masako Hasebea, Masaru Tomitaa, and Hiroshi Yanagawaa,b. (2009). Systematic identification of cell cycle-dependent yeast nucleocytoplasmic shuttling proteins by prediction of composite motifs. PNAS, vol. 106, no. 25, 10171–10176.

[22] Jyoti Chaudhary, Tinku Gautam, Vijay Gahlaut, Kalpana Singh, Sourabh Kumar, Ritu Batra, Pushpendra Kumar Gupta. (2022). Identification and characterization of RuvBL DNA helicase genes for tolerance against abiotic stresses in bread wheat (Triticum aestivum L.). Functional & Integrative Genomics. https://doi.org/10.1007/s10142-023-01177-y.

[23] Louise N. Johnson* and Richard J. Lewis. (2001). Structural Basis for Control by Phosphorylation. Chem. Rev, 101, 2209−2242.

[24] Gething, M. J. and Sambrook, J. (1992). "Protein folding in the cell." Nature, 355(6355), 33–45.

[25] Bjellqvist, B., Hughes, G. J., Pasquali, C., Paquet, N., Ravier, F., Sanchez, J. C. and Hochstrasser, D. F. (1993). "The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences." Electrophoresis, 14(1), 1023–1031.

[26] Heinemann, Björn. (2021). Amino acid metabolism under drought stress in Arabidopsis thaliana. Plant, Cell & Environment, Vol. 42, Issue 5, Pages 1630-1644 DOI: 10.1111/pce.13518.

[27] Tsuboyama, K., Tadakuma, H., Ishihama, Y., & Funatsu, T. (2018). "Cargo-mRNA stability controls localization and translation in neuronal RNA granules." Nature Communications, 9(1), 343.