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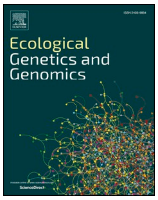

Bioinformatic analyses of microRNA profiles and protein-protein interactions within leaves from *Haberlea rhodopensis* subjected to severe drought

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ABSTRACT

Drought is a major threat to crop production, and resurrection plants provide a unique model for understanding extreme desiccation tolerance. While Apostolova et al. (2020) identified microRNAs (miRNAs) responsive to dehydration in *Haberlea rhodopensis*, the downstream protein targets and regulatory interaction networks remain largely unknown. This study addresses that gap by integrating miRNA profiles with protein–protein interaction (PPI) networks to predict target genes, identify hub regulators, and uncover pathways that regulate desiccation tolerance. Using PsRNATarget to predict mRNA targets (converted to *Arabidopsis thaliana* homologs) and Cytoscape-based network analysis, we identified three hub genes (SPL7 AT5G43270.3, and AT1G76580) as central regulators of the drought response. Functional enrichment revealed that metabolic pathways and secondary metabolite biosynthesis were significantly overrepresented, highlighting processes critical for survival under water deficit. This integrative approach moves beyond miRNA identification to demonstrate, for the first time, how miRNA-mediated regulation intersects with protein networks to coordinate desiccation tolerance in *H. rhodopensis*. The identified hub genes (SPL7 AT5G43270.3, and AT1G76580) can be utilized for future genetic engineering of drought-tolerant crops.

1. Introduction

Drought is a major problem worldwide, contributing up to 67% of crop and yield losses [1]. While most higher plants lose viability once they lose 41–70% of their total water content, resurrection plants have evolved the unique capacity to survive extreme desiccation, losing up to 95% of their water content and recovering rapidly upon rehydration [2]. *Haberlea rhodopensis*, a resurrection species endemic to the Balkans, has become an important model for understanding the molecular basis of desiccation tolerance [3,4].

MicroRNAs (miRNAs) are small, non-coding RNAs that play critical roles in the post-transcriptional regulation of gene expression, regulating plant development, stress signaling, and adaptation to stressful environments [5]. A pioneering study by Apostolova et al. (2020) provided the first genome-wide miRNA profile of *H. rhodopensis* under severe drought conditions, revealing families such as miR156/157 and miR399 as central to the desiccation response [2]. While this established a crucial regulatory mechanism, the study remained largely descriptive, focusing on differential expression without clarifying the downstream protein networks or functional pathways controlled by these miRNAs which are now addressed by this study.

Recent advances in genomic and transcriptomic studies of resurrection plants have revealed extensive reprogramming of photosynthesis, metabolism, and stress-response pathways during desiccation [1,6,7]. In addition, network biology approaches have emerged as powerful tools for identifying key regulators. For instance, Shoko et al. [8] applied protein–protein interaction (PPI) network analysis to the nuclear proteome of *Xerophyta schlechteri*, identifying heat shock proteins and ribosomal proteins as central hubs. While both our study and theirs employ similar bioinformatic approaches, there are important distinctions. Shoko's previous study was proteome-driven, beginning with experimentally derived protein datasets, whereas this study is the first to build PPI networks in *H. rhodopensis* starting from miRNA data [2]. This unique starting point allowed us to connect the regulatory mechanism of miRNAs with the functional proteomic analysis of hub genes and interaction networks, thereby offering a more integrated systems-level of desiccation tolerance.

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Acronyms

DEPs	Differentially Expresses Proteins
GO	Gene Ontology
PPIs	Protein-Protein Interactions
PsRNATarget	Plant small RNA Target

Table 1
MiRNA that were up-regulated and miRNA that were down-regulated during severe drought within leaves of *H. rhodopensis*.[a].

	MiRNA	Sequence
1	hrh-miR156a-5p	TTGACAGAAGATAGAGAGCAC
2	hrh-miR156b-5p	TGACAGAAGAGAGTGAGCACA
3	hrh-miR156c-5p	TTGACAGAAGATAGATAGCAC
4	hrh-miR157-5p	TGACAGAAGATAGAGAGCAC
5	hrh-miR157-3p	GCTCTCTATGCTTCTGTCATC
6	hrh-miR159a-3p	CTTGGACTGAAGGGAGCTCCC
7	hrh-miR168-5p	TCGCTTGGTGCAGGTCGGG
8	hrh-miR169-5p	CAGCCAAGGATGACTTGCC
9	hrh-miR171b-3p	TGAGCCGAATCAATATCACTC
10	hrh-miR396-5p	TTCCACAGCTTTCTTGAACT
11	hrh-miR5139-5p	AAACCTGGCTCTGATACCA
12	hrh-miR8175-3p	GATCCCCGGCAACGGCGCCA
13	hrh-miR160-3p	GCGTATGAGGAGCCAAGCATA
14	hrh-miR399b-3p	TGCCAAAGGAGATTTGCCCCG
15	hrh-miR159b-3p	TTTGGATTGAAGGGAGCTCCT
16	hrh-miR160-3p	GCGTATGAGGAGCCAAGCATA
17	hrh-miR164-5p	TGGAGAAGCAGGGCACGTGCT
18	hrh-miR166-5p	GGAATGTTGTCTGGCTCGAGG
19	hrh-miR166-3p	TCGGACCAGGCTTCATTCCCT
20	hrh-miR167-5p	TGAAGCTGCCAGCATGATCTGA
21	hrh-miR171a-3p	TTGAGCCGCGCCAATATCACT
22	hrh-miR319-3p	CTTGGACTGAAGGGAGCTCCT
23	hrh-miR395-3p	CTGAAGTGTTTGGGGGAACTC
24	hrh-miR399a-3p	TGCCAAAGGAGAGTTGCCCTG
25	hrh-miR399b-3p	TGCCAAAGGAGATTTGCCCCG
26	hrh-miR399c-3p	TGCCAAAGGAGATTTGCCCCT
27	hrh-miR157-5p	TGACAGAAGATAGAGAGCAC
28	hrh-miR8175-3p	GATCCCCGGCAACGGCGCCA

[a] A total of 28 miRNAs were retrieved, the first 14 miRNAs were up-regulated and the last 14 miRNAs were down-regulated during severe drought conditions as shown in Table 1 above.

The primary objective of this study was therefore to advance beyond miRNA profiling by predicting their protein targets, constructing and analyzing PPI networks, identifying hub genes and functional modules, and performing enrichment analyses to reveal the critical biological pathways involved in desiccation tolerance. Due to the absence of a complete genome for *H. rhodopensis*, predicted miRNA targets were mapped to *Arabidopsis thaliana* homologs, a necessary but strategic choice given its well-annotated genome and close evolutionary relationship [3,4]. While this reliance represents a limitation, it ensures meaningful biological interpretation until comprehensive genomic resources for *H. rhodopensis* become available.

This study represents a logical and novel progression in the systems biology of resurrection plants. By integrating miRNA regulators with their downstream protein interaction networks, we were able to identify hub genes and functional pathways that contribute to desiccation tolerance in *H. rhodopensis*. These findings provide the first integrated miRNA–protein network framework for this species, complementing proteomic-centric studies in other resurrection plants. The superiority of this study lies in its ability to move beyond expression profiling to uncover functional modules and regulatory mechanisms, thereby offering not only deeper mechanistic insights but also specific candidate targets for engineering drought-tolerant crops.

2. Methods

In the absence of a fully sequenced and annotated genome for *Haberlea rhodopensis*, miRNA targets were predicted and subsequently mapped to *Arabidopsis thaliana* homologs to facilitate downstream network and enrichment analyses. *Arabidopsis thaliana* was chosen as the reference species owing to its well-curated genome, extensive functional annotation, and evolutionary relatedness, which provide a reliable framework for cross-species comparative studies [9,10]. Although this cross-species approach may introduce some uncertainty in gene annotation [7,11,12] it remains a necessary and widely adopted strategy until complete genomic resources for *H. rhodopensis* become available.

2.1. *Retrieval of miRNAs*

The miRNAs that were used in this study were retrieved from an article titled “MicroRNA profiling the resurrection plant *H. rhodopensis* unveils essential regulators of survival under severe drought” [2], and presented in Table 1.

2.2. *Prediction of miRNA targets*

The prediction of potential miRNA targets was carried out using PsRNATarget (2017 update), which identifies the binding sites by exploiting sequence complementarity between miRNAs and target mRNA sequences [13]. In the process of target prediction, the selected target transcript library was the Araport one-stop-shop for *Arabidopsis thaliana* genomics, meaning that the miRNA targets were converted to *A. thaliana* homologs. This is because there is scarce genomic data for *H. rhodopensis*, since there is limited research on this species as compared to the model organism (*A. thaliana)* [9,14].

2.3. *Construction of PPI networks*

We used GeneMANIA version 3.5.2 [15] plugged in Cytoscape version 3.9.1 to construct PPI networks [16], predict interactions among differentially expressed proteins (DEPs), and explore various biological relationships and associations within the networks using *A. thaliana*. Two PPI networks (up-regulated and down-regulated proteins) were constructed. Of note, gene IDs were used as the query. Self-loops and duplicate edges were excluded in each PPI network to enhance clarity. Furthermore, GeneMANIA aided in the analysis of relationships between proteins within the networks, including similarities in protein domains, and predicted interactions, co-expression patterns, physical and genetic interactions, pathways, and co-localization [15].

2.4. *Identification of hub genes*

Identification of hub genes was carried out through the assessment of node degree distribution utilizing the network analyzer application incorporated in the Cytoscape application [17].

2.5. *Construction of a sub-network using key genes*

From the main PPI networks, nodes with high degree distribution were regarded as crucial nodes [6]. Based on degree distribution, the top 20 nodes in each PPI network were regarded as key genes responsible for the plant's response to severe drought stress. These key genes from each PPI network were then assembled into a sub-PPI network, making it easier to carry out functional enrichment analysis.

2.6. *Identification of functional modules in the PPI networks using community structure analysis*

We constructed a network using all the DEPs (both up- and down-regulated proteins) and performed cluster analysis to identify densely

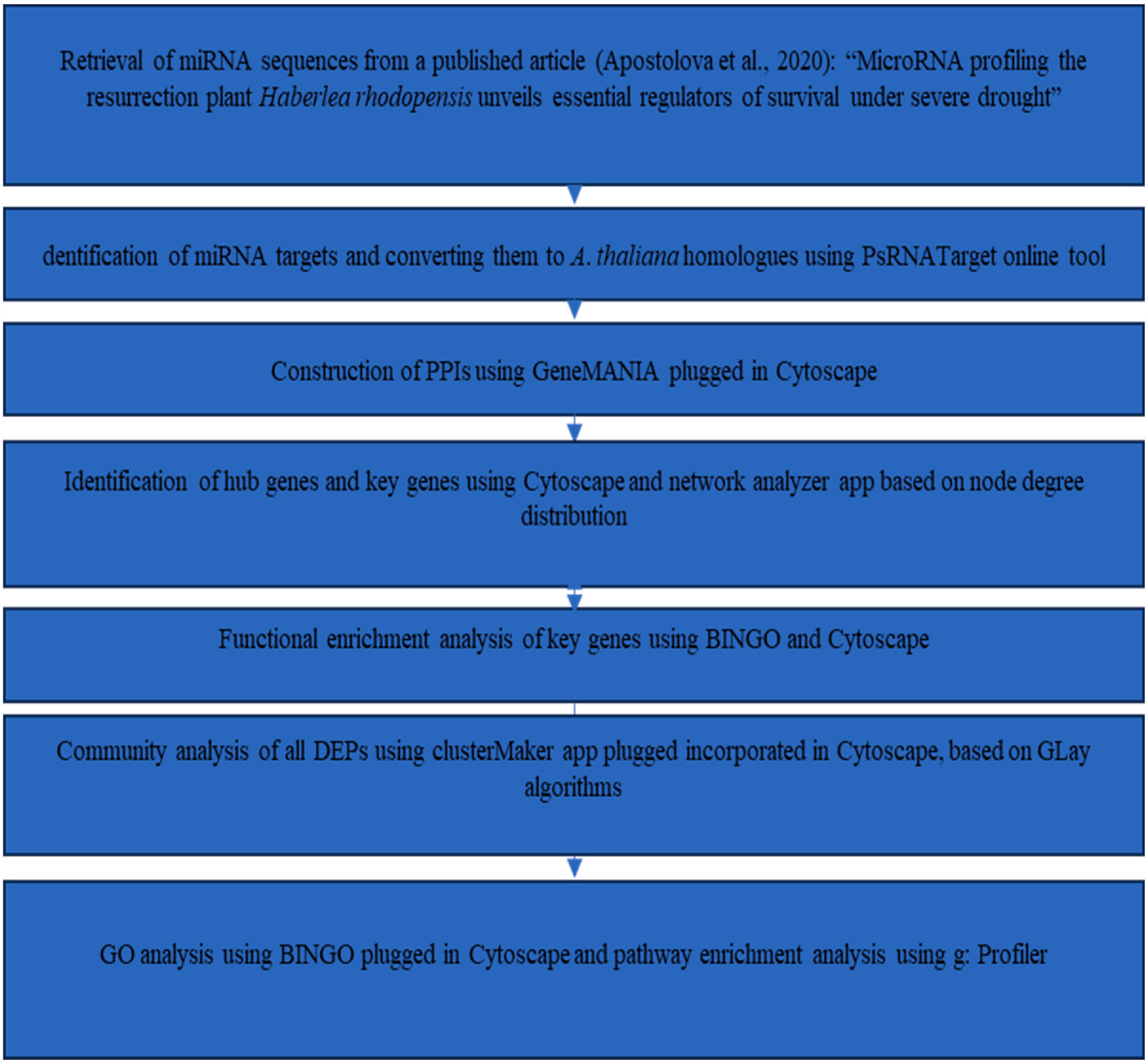

Fig. 1. An overview of the methods used in this study

The diagram above (Fig. 1) provides a concise overview of the methods that were used in this study.

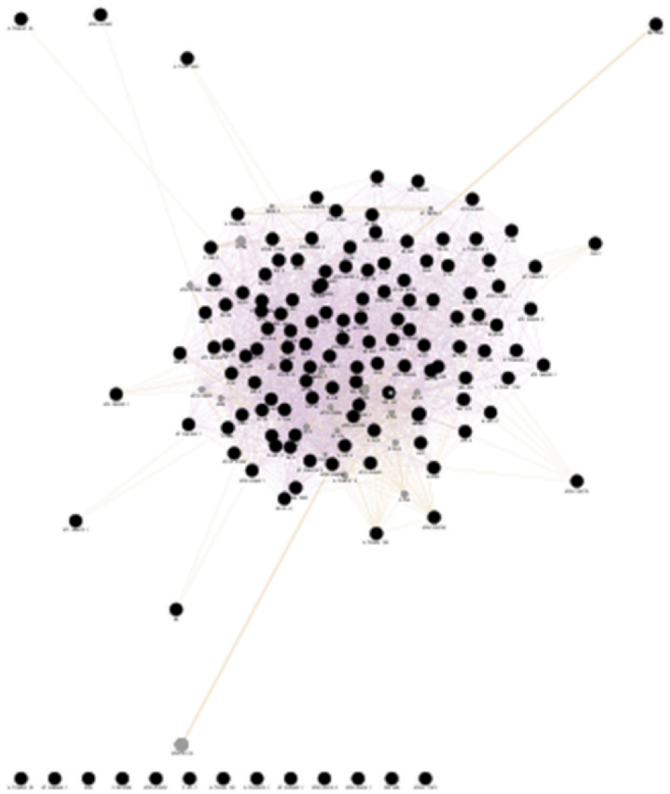

Fig. 2. The PPI network for the up-regulated proteins
The PPI network in Fig. 2 is composed of 5484 edges between 240 nodes based on co-expression (86.26%), shared protein domain (4.80%), predicted (3.75), co-localization (2.78%), and physical interactions (2.40); the average number of neighbours is 47.687.

connected regions. Community clustering analysis based on GLaY algorithm [18] in Cytoscape was carried out with clusterMaker2 v2.3.4 to detect functional modules among the DEPs.

Importantly, enrichment pathway analysis for each cluster was carried out using g: Profiler; *A. thaliana* was selected as the reference organism and gene IDs as the query. Only clusters with several nodes (clusters 14, 15, and 16) were analyzed. The analysis focused on annotated genes only within a statistical domain scope. A significance threshold of 0.05 was used, utilizing the g: SCS method.

2.7. *Functional enrichment analysis of key genes and clusters*

Functional enrichment analysis was carried out using BINGO v 3.0.5 [19], plugged in Cytoscape with default parameters to acquire better insights on the biological significance of the key proteins in each constructed PPI network. Gene names were used as the query, and *A. thaliana* was selected as the query organism. This research revealed significantly overrepresented Gene Ontology Biological Process (GO BP) terms. BINGO summarizes big lists of GO concepts by deleting redundant terms. In addition to GO BP, BINGO was also used to analyze molecular function terms and cellular component [19].

3. Results and discussion

3.1. *MicroRNA regulation in response to drought stress*

A total of 28 miRNAs were identified, with 14 miRNAs up-regulated and 14 miRNAs down-regulated during severe drought conditions. Key miRNAs identified include miR156/157 and miR399, which play a crucial role in regulating desiccation-responsive genes. The miRNA-mediated regulation of gene expression has an effect on various biological processes, including transcriptional regulation, hormone signaling, and nutrient uptake which enhance desiccation tolerance [20].

3.2. *PPI networks of DEPs*

PPI networks were constructed to understand the functional organization and regulation of cellular processes in response to severe drought stress [7]. Black nodes denoted the query genes and additional proteins harnessed by GeneMANIA were represented as gray nodes as shown in Figs. 2–5.

The PPI network in Fig. 2 is a relatively dense and interconnected network, with different types of interactions, co-expression is the most prominent, and accounting for 86.26% of the interactions indicating that these up-regulated genes/proteins may share common regulatory mechanisms and work together to respond to drought stress. Shared protein domain interactions contribute to 4.80% of the interactions, indicating functional similarities between these proteins. Predicted interactions, co-localization, and physical interactions make up smaller proportions, suggestive of additional regulatory and physical associations [7].

Similarly, the PPI network for down-regulated proteins as shown in Fig. 3 exhibited coordinated regulation and functional similarities. Co-expression interactions dominated, and shared protein domain interactions were significant. Predicted interactions, physical interactions, and co-localization played smaller roles [21].

3.2.1. *Sub-PPI interaction network of key proteins*

PPIs in *H. rhodopensis* provide valuable information about their functional relationships and potential roles in the plant's response to drought stress. Similar to the up-regulated network, co-expression interactions dominate, accounting for 88.49% of the interactions. This suggests coordinated regulation of down-regulated genes/proteins in response to drought. Shared protein domain interactions contribute significantly more in this network, comprising 8.67% of the interactions. The networks highlight the importance of co-expression and shared protein domains, indicating coordinated regulation and functional similarities among these genes/proteins, offering potential candidates for further investigation into the molecular mechanisms underlying drought response in this plant species [21].

3.2.2. *Identification of key genes using node degree distribution*

Node degree distribution was used to identify key genes/proteins in the PPI network. Nodes with higher degrees (more connections) were considered more influential, indicating their potential significance in biological processes [22,23]. This analysis identified several high-degree hub genes from both the up-regulated and down-regulated networks (Tables 2 and 3).

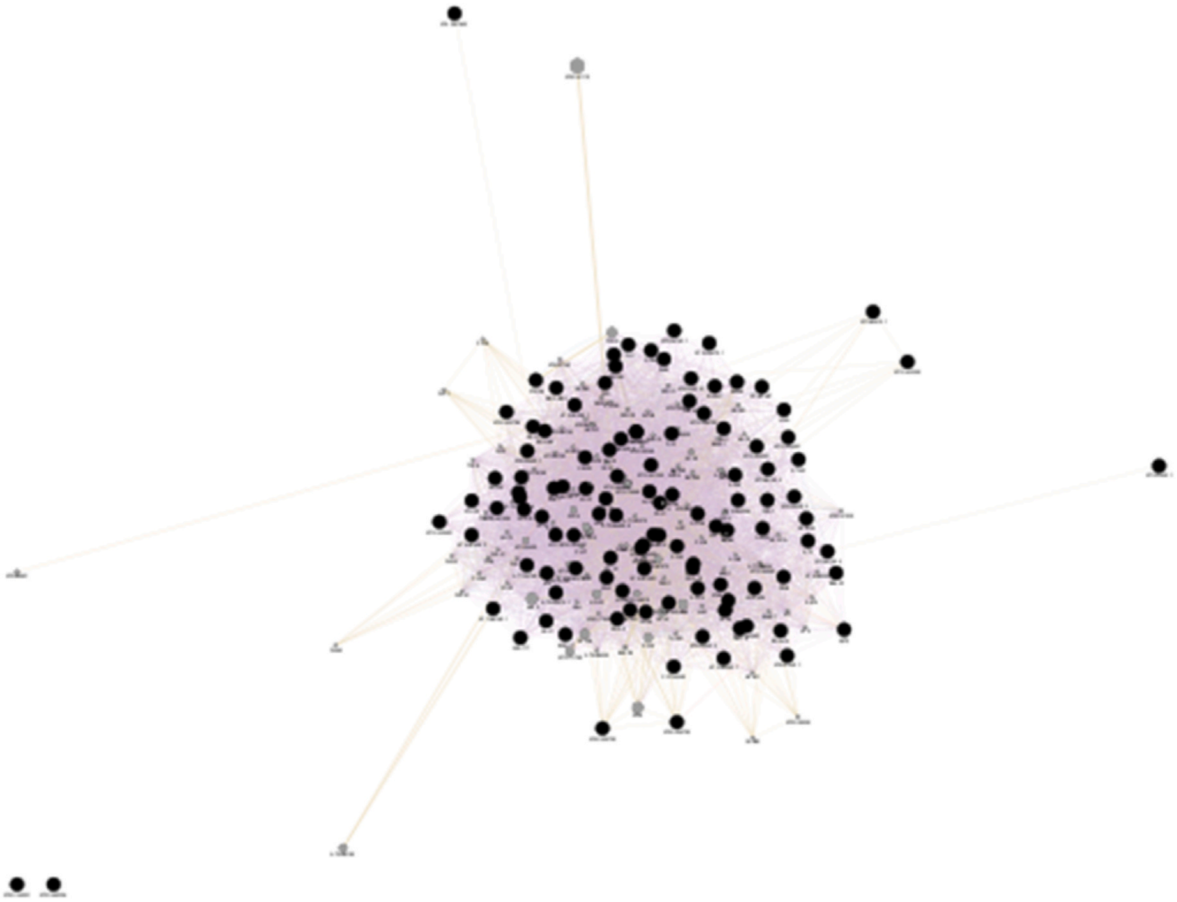

Fig. 3. The PPI network for the down-regulated proteins
The PPI network shown in Fig. 3 is composed of 5176 edges between 213 nodes based on co-expression (88.49%), shared protein domain (8.67%), predicted (1.65), physical interactions (1.07%), and co-localization (0.12%); the average number of neighbours is 49.062.

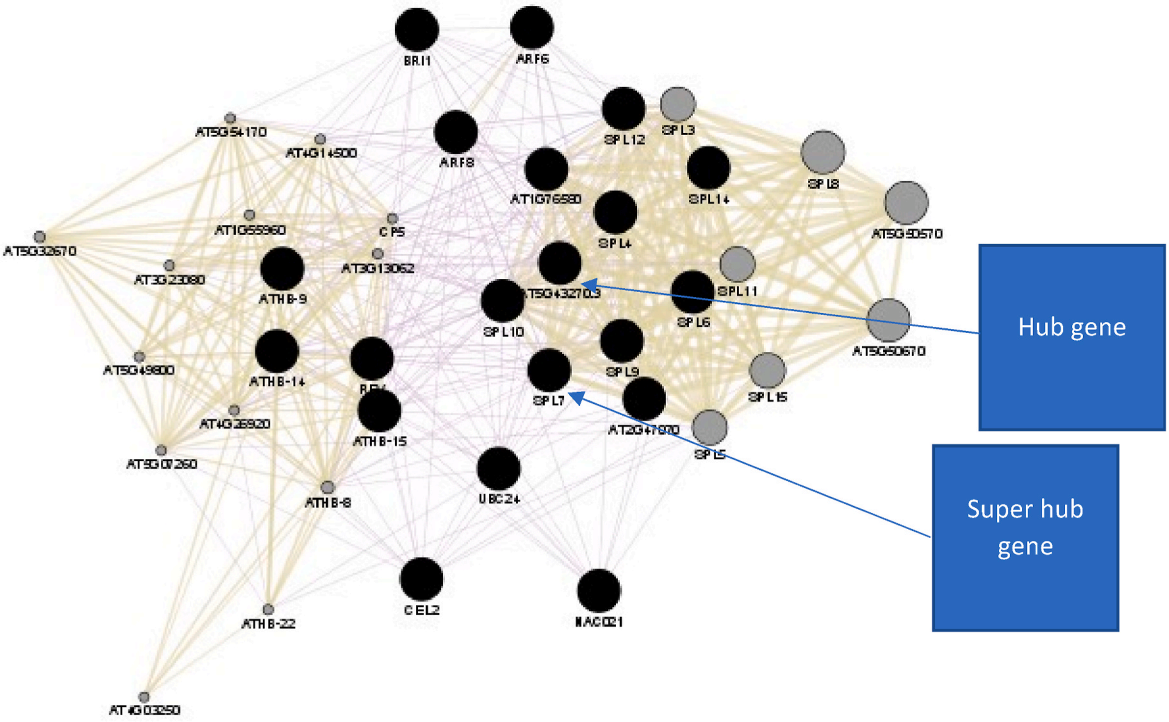

Fig. 4. PPI sub-network of key proteins for proteins that were up-regulated
The above PPI network as shown 3 is composed of 423 edges between 40 nodes based on co-expression (62.39%), shared protein domain (37.22%), and co-localization (0.40%). The average number of neighbours is 21.150.

The most significant up-regulated hub genes, SPL7 and AT5G43270.3 (Table 2), are critically involved in stress adaptation. Squamosa Promoter-Binding-Like Protein 7 (SPL7) is a key regulator of copper homeostasis, which is essential for the activation of antioxidant enzymes such as copper/zinc superoxide dismutase to mitigate oxidative damage during desiccation [10]. It also contributes to the regulation of stomatal movement, aiding in water conservation [24]. While AT5G43270.3 is currently uncharacterized, its high connectivity with known stress-responsive factors like SPL7 marks it as a critical node, suggesting a significant, yet-to-be-defined role in maintaining cellular homeostasis under drought stress [25].

Conversely, key down-regulated hubs included SPL7 and AT1G76580 (Table 3). The presence of SPL7 as a hub in both networks indicates complex, condition-specific regulation, where different SPL7-mediated pathways may be selectively activated or suppressed to fine-tune the stress response. The down-regulation of AT1G76580, a highly connected uncharacterized protein, suggests its suppression is a coordinated event to shut down processes detrimental under drought, potentially involving the regulation of aquaporin activity and water balance [11], or regulating abscisic acid signaling to enhance the expression of other drought-responsive genes [26].

Apart from hub genes, other genes in Tables 2 and 3 further facilitate the coordinated cellular responses to drought stress. For instance, the up-regulation of UBC24 (a target of miR399) suppress phosphate uptake and metabolism to conserve resources [2]. The central role of homeodomain transcription factors (ATHB-14, ATHB-9, ATHB-15) and auxin response factors (ARF6, ARF8), as indicated by their high node degrees [22,23], underscores their function in regulating a major reprogramming of developmental and growth pathways. Similarly, the down-regulation of multiple NFYA transcription factors (Table 3) indicates reprogramming of gene expression away from standard growth and towards a specialized, stress-tolerant state [27].

The genes identified in Tables 2 and 3 collectively regulate complex response encompassing transcriptional reprogramming, oxidative stress mitigation, nutrient homeostasis, and hormonal signaling, which collectively enhance the extreme desiccation tolerance of *H. rhodopensis.*

3.2.3. Functional enrichment of key genes

The GO cellular function for the up-regulated genes included the cell, cell part, intracellular, intracellular part, organelle, intracellular organelle, membrane-bound organelle, and nucleus refer to the txt document in the appendices. These GO terms show the cellular processes and organelles involved in enhancing desiccation tolerance in *H. rhodopensis* [3,28,29].

The molecular function ontologies include binding, nucleic acid binding, transcription regulator activity, transcription factor activity and cellulose activity. These molecular function ontologies collectively contribute to the resilience of *H. rhodopensis* against desiccation by regulating gene expression, maintaining cellular integrity, and ensuring proper cellular responses to stress conditions [4,30].

The GO biological function as shown in appendix 1 for the up-regulated key genes include response to stimulus, biological regulation, regulation of the biological; cellular; metabolic; developmental; multicellular organismal-; macromolecule metabolic; nitrogen compound metabolic; primary metabolic; cellular biosynthetic; and nucleobase, nucleoside, nucleotide, and nucleic acid metabolic processes, and gene expression. The up-regulation of key genes associated with these GO biological functions collectively enhances desiccation tolerance in *H.*

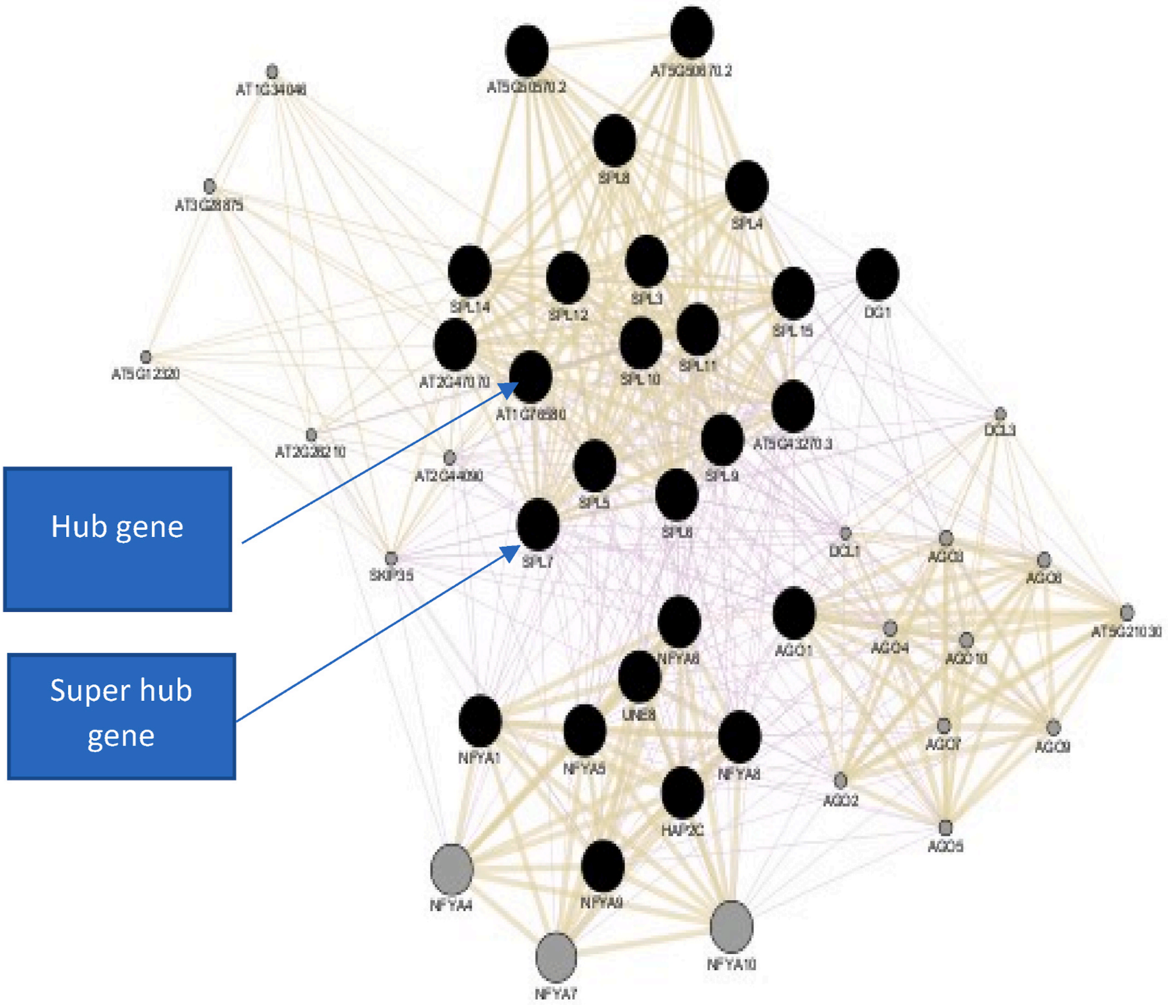

Fig. 5. PPI sub-network of key proteins for proteins that were down-regulated
The above PPI network is composed of 481 edges between 46 nodes based on co-expression (31.93%), shared protein domain (67.62%), and predicted (0.46%). The average number of neighbours is 20.913.

rhodopensis by regulating stress responses, maintaining cellular functions, and ensuring adaptive mechanisms are activated during water deficiency [4,28].

Down-regulated genes were associated with reduced metabolic activity during desiccation to decrease water loss and increase cellular stability, contributing to the plant's desiccation tolerance [11,31,32] as shown in appendix 2. The GO ontologies for cellular components include cell, cell part, intracellular, intracellular part, membrane-bound organelle, intracellular membrane-bound organelle, nucleus and CCAAT-binding factor complex. The GO molecular function of the key genes that were down-regulated include binding, nucleic acid binding, DNA binding, transcription factor regulator activity and transcriptional factor activity. The GO biological process associated with key genes that were down-regulated include biological regulation, regulation of the following biological process, metabolic process, developmental process, macromolecule metabolic process, transcription, multicellular organismal process, gene expression, cellular biosynthetic process, nitrogen compound metabolic process, multicellular organismal development, and anatomical structure development. These cellular components collectively contribute to the plant's ability to withstand desiccation by ensuring structural integrity, efficient water management, and appropriate gene regulation responses [27,33].

Table 2
Key genes/proteins that were up-regulated as predicted by GeneMANIA.[a].

Gene name	Description	Degree
SPL7*	Squamosa promoter-binding-like protein 7	62
AT5G43270.3*	AT5G43270.3: Uncharacterized protein At5g43270.3	58
REV	Reversal of embryo defective	57
UBC24	Ubiquitin-conjugating enzyme 24	55
ATHB-14	*Arabidopsis thaliana* homeobox 14	54
ARF8	Auxin response factor 8	54
AT1G76580	Uncharacterized protein At1g76580	54
SPL14	Squamosa promoter-binding-like protein 14	51
SPL6	Squamosa promoter-binding-like protein 6	51
ATHB-9	*Arabidopsis thaliana* homeobox 9	50
SPL12	Squamosa promoter-binding-like protein 12	50
ATHB-15	*Arabidopsis thaliana* homeobox 15	50
SPL10	Squamosa promoter-binding-like protein 10	49
CEL2	Cellulose synthase-like protein 2	48
BRI1	Brassinosteroid-insensitive 1	48
SPL4	Squamosa promoter-binding-like protein 4	48
NAC021	NAC domain-containing protein 021	47
ARF6	Auxin response factor 6	47
SPL9	Squamosa promoter-binding-like protein 9	47
AT2G47070	AT2G47070: Uncharacterized protein At2g47070	47

[a] Table 2 shows a list of key genes that were up-regulated in response to severe drought. SPL7* and AT5G43270.3* are hub genes with high node degree distribution of 62 and 58, respectively.

Table 3
Key down-regulated genes/proteins as predicted by GeneMANIA.[a]

Gene name	Description	Degree
SPL7*	Squamosa promoter-binding-like protein 7	27
AT1G76580*	Uncharacterized protein At1g76580	27
AT5G43270.3	Uncharacterized protein At5g43270.3	26
SPL10	Squamosa promoter-binding-like protein 10	26
SPL12	Squamosa promoter-binding-like protein 12	26
SPL6	Squamosa promoter-binding-like protein 6	25
SPL4	Squamosa promoter-binding-like protein 4	25
SPL11	Squamosa promoter-binding-like protein 11	24
SPL14	Squamosa promoter-binding-like protein 14	24
SPL3	Squamosa promoter-binding-like protein 3	23
SPL15	Squamosa promoter-binding-like protein 15	22
NFYA6	Nuclear factor Y, subunit A6	22
SPL9	Squamosa promoter-binding-like protein 9	20
HAP2C	Homeodomain-like transcription factor HAP2C	19
DG1	Drought-induced 1	18
AT2G47070	Uncharacterized protein At2g47070	18
SPL5	Squamosa promoter-binding-like protein 5	17
AT5G50670.2	Uncharacterized protein At5g50670.2	17
NFYA1	Nuclear factor Y, subunit A1	16
NFYA9	Nuclear factor Y, subunit A9	16
NFYA5	Nuclear factor Y, subunit A5	16
AGO1	Protein ARGONAUTE 1	16
UNE8	Ethylene-responsive transcription factor UNE8	16
SPL8	Squamosa promoter-binding-like protein 8	16
NFYA8	Nuclear factor Y, subunit A8	16
AT5G50570.2	Uncharacterized protein At5g50570.2	16

[a] Table 3 shows a list of key genes that were down-regulated in response to severe drought. SPL7* and AT1G76580* are hub genes with a high node distribution of 27.

3.3. *Identification of functional modules in the PPI network using community structure analysis*

Genes were organized into clusters as shown in Fig. 6 in order to assess their biological pathways effectively [34]. These pathways as shown in appendices 6-8, collectively contribute to desiccation tolerance by orchestrating several mechanisms such as stress response, metabolic adjustments, and signaling which enable the *H. rhodopensis* to survive under extreme dehydration conditions [32].

Functional enrichment analysis shows that cluster 14 (appendix 3) is enriched for nuclear and transcriptional regulation components and functions, including transcription factor activity, transcription regulation, nucleic acid binding, and processes involved in metabolic regulation, gene expression, and development. Cluster 15 (appendix 4) is enriched for membrane-bound organelles, DNA binding, transcriptional regulation, and processes related to metabolism, biosynthesis, gene expression, and cellular function, highlighting the importance of regulatory mechanisms in desiccation stress response [4,30]. Cluster 16 as shown in appendix 5 is enriched for the F-actin capping complex, suggesting a role in cytoskeletal organization and cellular structure maintenance [12], as well as protein binding, DNA binding, and transport functions, along with regulatory, developmental, and anatomical processes that are crucial for adapting to desiccation stress [24,35,36].

4. Conclusion

This study revealed insights into the molecular mechanisms underlying desiccation tolerance in *H. rhodopensis* which might be useful in genetic engineering of desiccation-tolerant crops and breeding technologies to promote sustainable agriculture in water-deficit environments. According to the findings, miRNAs regulate desiccation tolerance mechanisms in *H. rhodopensis*. Key miRNAs identified include miR156/157 and miR399, which play a crucial role in regulating desiccation-responsive genes. SPL7, AT5G43270, and AT1G76580 were identified as hub genes. Metabolic pathways and biosynthesis of secondary metabolites, were the main pathways which contribute to desiccation tolerance.

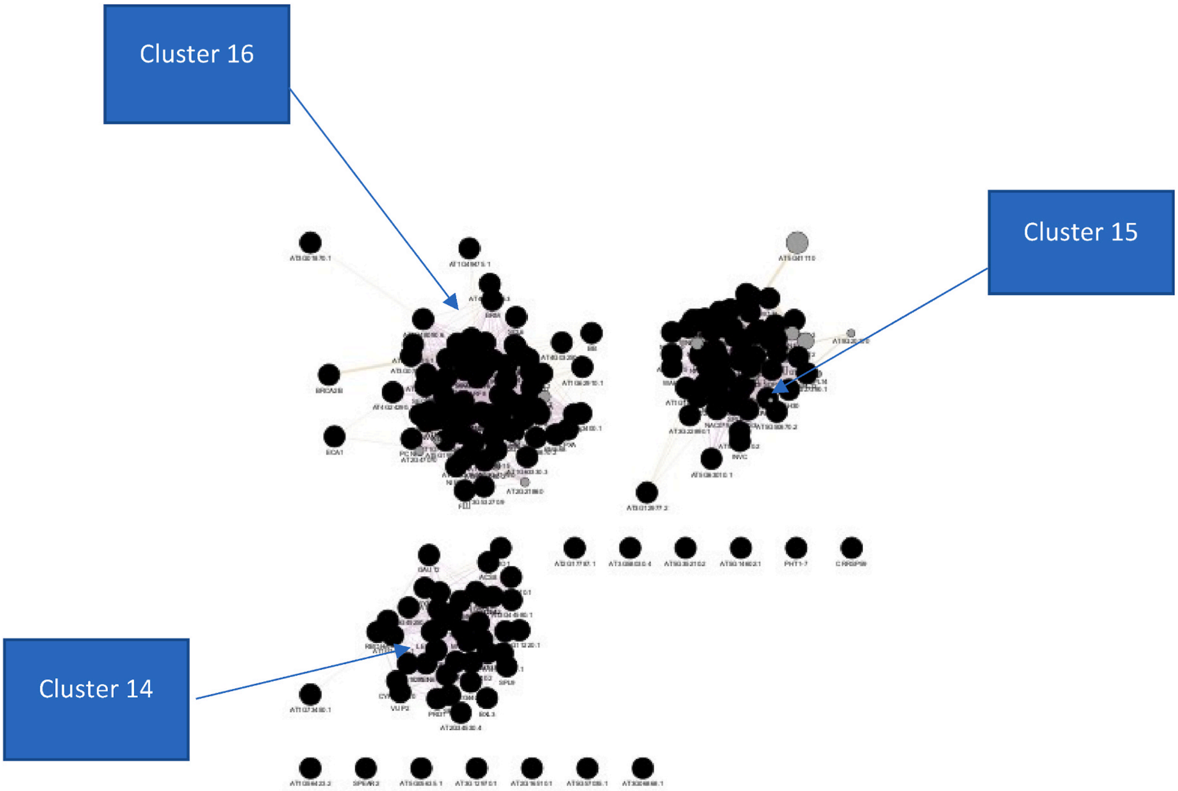

Fig. 6. An illustration of the clusters formed by the fast greedy (GLay) clustering algorithm, consisting of all differentially expressed genes.

4.1. Limitations and recommendations

The targets for miRNA were converted to *A. thaliana* homologs (assuming species homology); however, there may be species-specific differences in miRNA targeting and protein interactions. Furthermore, bioinformatic tools and algorithms depend on existing databases and annotations, which may have biases or incomplete information, hence there is need for experimental (wet-lab) validation of the results. Regulatory mechanisms other than microRNA, such as other small interfering RNA, chromatin remodeling and epigenetic modifications must be taken into consideration because they might also contribute to desiccation tolerance in *H. rhodopensis*.

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CRediT authorship contribution statement

Catherine Fundikwa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Kudzai B. Nyazema:** Supervision, Visualization, Writing – review & editing. **Ryman Shoko:** Data curation, Formal analysis, Methodology, Software, Supervision, Writing – review & editing.

Declaration of competing interest

I, Catherine Fundikwa, declare that I have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.egg.2026.100477.

Data availability

I have uploaded all the data during submission

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