Journal of Tropical Biodiversity and Biotechnology
Volume 11, Issue 01 (2026): jtbb17108
DOI: 10.22146/jtbb.17108

Research Article

Characterisation of CONSTANS Gene Family from the Amaranthaceae Family and Their Response to Adverse Environmental Conditions Based on Genome-Wide Identification and Transcriptome Analysis

Xuan Duong Vu[1], Phi Bang Cao[2], Quy Phuong Nguyen[2], Thi Thanh Huyen Tran[3], Hong Viet La[4], Duong Ngoc Dao[2,5], Quynh Thi Ngoc Le[6], Ha Duc Chu[7]*

1)Institute of Applied Research and Development, Hung Vuong University, Phu Tho Province 35000, Vietnam
2)Faculty of Natural Sciences, Hung Vuong University, Phu Tho Province 35000, Vietnam
3)Faculty of Biology, Hanoi National University of Education, Xuan Thuy Road, Hanoi City 122300, Vietnam
4)Institute of Research and Application, Hanoi Pedagogical University 2, Phu Tho Province 280000, Vietnam
5)Que Lam Junior High School, Doan Hung, Phu Tho Province 35300, Vietnam
6)Department of Biotechnology, Thuyloi University, Hanoi City 116830, Vietnam
7)Faculty of Agricultural Technology, University of Engineering and Technology, Vietnam National University Hanoi, Hanoi City 122300, Vietnam
* Corresponding author, email: cd.ha@vnu.edu.vn

Keywords:
CONSTANS
Transcription factor
Amaranthus
Identification
Characterisation
Gene structure
Expression pattern
Submitted:
19 October 2024
Accepted:
17 July 2025
Published:
13 February 2026
Editors:
Furzani Binti Pa'ee
Sri Nopitasari

ABSTRACT

The CONSTANS (CO) transcription factor plays a key role in regulating photoperiodic flowering by integrating environmental signals and activating downstream flowering genes. However, no comprehensive information on the CO transcription factor family has been reported in *Amaranthus* species. In this study, we performed a genome-wide identification, characterisation, and expression analysis of the CO gene family in six *Amaranthus* species, including *A. hypochondriacus*, *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor*. A total of 12 to 13 *CO* genes were identified in each species, and their chromosomal positions, gene structures, and physicochemical properties were analysed. Phylogenetic analysis categorised these *CO* genes into three distinct groups, revealing evolutionary relationships with *CO* genes from *Arabidopsis thaliana* and sugar beet. Gene structure analysis showed considerable diversity in exon-intron organisation, indicating functional differentiation within the CO family. Additionally, transcriptome analysis using RNA-Seq data demonstrated tissue-specific and stress-induced expression patterns, particularly under drought and herbicide treatments. This study provides the first comprehensive insight into the CO transcription factor family in *Amaranthus* species, offering a foundation for future research on photoperiodic flowering regulation and stress-response mechanisms in these species.

How to cite:
Vu, X.D. et al., 2026. Characterisation of CONSTANS Gene Family from the Amaranthaceae Family and Their Response to Adverse Environmental Conditions Based on Genome-Wide Identification and Transcriptome Analysis. *Journal of Tropical Biodiversity and Biotechnology*, 11(1), jtbb17108. doi: 10.22146/jtbb.17108

INTRODUCTION

The Amaranthaceae family, commonly known as the Amaranth family, comprises a diverse group of globally distributed plants with significant agricultural and ecological importance. This family includes *Amaranthus hypochondriacus*, *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor*. Originating primarily from the Americas, many of these species have been cultivated for their nutritional value since ancient times (Caselato-Sousa & Amaya-Farfán 2012). For instance, *A. hypochondriacus* and *A. cruentus* are renowned for their high-protein grains and are staple crops in various cultures, while *A. tricolor* is widely grown in Asia as a leafy vegetable rich in vitamins and minerals (Soriano-García & Aguirre-Díaz 2019; Baraniak & Kania-Dobrowolska 2022). On the other hand, *A. palmeri* and *A. tuberculatus* are notable for their adaptability and have become pervasive agricultural weeds that challenge crop production due to their resistance to common herbicides. Understanding how these plants sense photoperiod information to predict impending environmental changes and precisely regulate flowering time under favourable conditions at the molecular level is essential (Pulvento & Sellami 2021). Investigating these mechanisms will not only advance our understanding of plant developmental biology but also provide practical benefits for improving crop yields, controlling weed species, and adapting agricultural practices to the challenges of climate change (Roeber et al. 2021b; Jan et al. 2023). Moreover, insights gained from such studies could contribute to broader agricultural advancements by informing breeding programmes aimed at improving stress resilience and productivity in other crop species (Roeber et al. 2021a; Baguma et al. 2023).

CONSTANS (CO) is a pivotal transcription factor in plants that plays a central role in regulating photoperiodic flowering by integrating environmental light cues with the plant's internal circadian clock (Kim et al. 2008; Zhang et al. 2023). Structurally, CO is characterised by two conserved B-box zinc finger domains at its N-terminus, which are involved in protein-protein interactions and may contribute to DNA-binding specificity (Dahal et al. 2022). These domains are crucial for forming functional protein complexes that regulate downstream genes (Dahal et al. 2022; Zhang et al. 2023). At its C-terminus, CO contains a CONSTANS, CO-like, and TOC1 domain, which is essential for nuclear localisation and mediates interactions with other circadian clock components (Wenkel et al. 2006). Functionally, CO acts as a transcriptional activator of the *FLOWERING LOCUS T* gene, promoting the transition from vegetative growth to flowering in response to favourable photoperiod conditions, particularly under long-day environments (Zhang et al. 2023). As a key gene in the photoperiod pathway (Kim et al. 2008; Zhang et al. 2023), understanding the CONSTANS transcription factor in *A. hypochondriacus* is necessary to elucidate how this species perceives and responds to photoperiodic signals at the molecular level. To date, the CO transcription factor family has been investigated in various plant species, including barley (*Hordeum vulgare*) (Griffiths et al. 2003), rice (*Oryza sativa*) (Griffiths et al. 2003), Arabidopsis (*Arabidopsis thaliana*) (Griffiths et al. 2003), mango (*Mangifera indica*) (Liu et al. 2022), Chinese white pear (*Pyrus bretschneideri*) (Wang et al. 2017), grapevine (*Vitis vinifera*) (Wang et al. 2019), sugar beet (*Beta vulgaris*) (Chia et al. 2008), *Chrysanthemum lavandulifolium* (Fu et al. 2015), gingko (*Ginkgo biloba*) (Yan et al. 2017), and quinoa (*Chenopodium quinoa*) (Tran et al. 2025). However, there is no report on the CO transcription factor family in *A. hypochondriacus*.

Studying CO in this context will shed light on the unique regulatory mechanisms employed by *A. hypochondriacus* and contribute to optimising flowering time and enhancing agronomic traits in this nutritionally important and stress-resilient crop. Such insights are crucial for enhancing yield, adapt-

ing cultivation practices to shifting climatic conditions, and ultimately contributing to global food security.

This study aims to conduct a comprehensive genome-wide identification, characterisation, and expression analysis of the CO transcription factor family in six *Amaranthus* species. By investigating their gene structure, evolutionary relationships, physicochemical properties, and expression patterns across various tissues and environmental conditions, this study seeks to provide insights into the functional roles of *CO* genes in photoperiodic flowering and stress responses. The findings aim to enhance our understanding of the CO transcription factor family in the Amaranthaceae family and contribute to future crop improvement strategies.

MATERIALS AND METHODS

Database search of *CO* genes

To identify *CO* genes within the Amaranthaceae family (Singh et al. 2023), we conducted a protein-based search strategy. Firstly, well-known CO protein sequences from *A. thaliana* (Griffiths et al. 2003) were obtained from TAIR (http://www.arabidopsis.org/) and used as queries in a local BLASTP search against the predicted proteomes of six *Amaranthus* species, including *A. hypochondriacus* (Sunil et al. 2014), *A. palmeri* (Montgomery et al. 2020), *A. cruentus* (Ma et al. 2021), *A. hybridus* (Montgomery et al. 2020), *A. tuberculatus* (Montgomery et al. 2020), and *A. tricolor* (Wang et al. 2023). All predicted proteins were then analysed using HMMER (https://www.ebi.ac.uk/Tools/hmmer/) (Potter et al. 2018) with a conserved CO domain downloaded from the Pfam database (http://pfam.xfam.org/) (Mistry et al. 2021), applying an E-value threshold between 0.0001 and 0.001. The filtered protein sequences were designated as CO transcription factors of Amaranthaceae species for further evaluation.

Characterisation of CO transcription factors

To analyse the physicochemical characteristics of CO transcription factors, we employed the ProtParam tool available on the ExPASy server (Gasteiger et al. 2003, 2005) as previously described (La et al. 2022; H.D. Chu et al. 2024). The amino acid sequences of the CO proteins were retrieved from the proteome assemblies of the respective *Amaranthus* species and input into ProtParam (Gasteiger et al. 2003, 2005) for analysis. This tool calculated various physicochemical parameters, including molecular weight, theoretical isoelectric point, aliphatic index, and grand average of hydropathicity.

Categorisation of CO transcription factors

To investigate the evolutionary relationships of CO transcription factors among six *Amaranthus* species (*A. hypochondriacus*, *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor*), we constructed a phylogenetic tree using the Maximum Likelihood method as previously described (La et al. 2022; H.D. Chu et al. 2024). Multiple sequence alignment was performed using ClustalW (Thompson et al. 2002; Larkin et al. 2007) to obtain the alignment of conserved domains. The aligned sequences were then used to build the phylogenetic tree employing the Maximum Likelihood approach implemented in MEGA X software (Kumar et al. 2018). To assess the statistical support of the phylogenetic groupings, a bootstrap analysis with 1000 replicates was conducted as previously described (Cao 2022; La et al. 2022; N.T.B. Chu et al. 2024).

Structural analysis of *CO* genes

To analyse the gene structure of the CO gene family in *A. hypochondriacus*, we employed the GSDS tool (Hu et al. 2015) as previously described (Chu et al.

2018; Tran et al. 2025). The exon-intron arrangements were visualised by comparing full-length genomic sequences with their corresponding coding sequences. First, the phylogenetic tree of the CO gene family in *A. hypochondriacus* was constructed to guide the structural arrangement, ensuring that genes from closely related clades were analysed in a comparative context. The input genomic and coding sequences were aligned, and the GSDS tool (Hu et al. 2015) was used to map the distribution and number of exons and introns in each gene.

Transcriptome analysis of *CO* genes

To analyse the transcriptome of *CO* genes in *Amaranthus* species, recent RNA -Seq datasets reported in previous studies (Clouse et al. 2016) were obtained from the Phytozome (Goodstein et al. 2012) and AGRDB databases (Singh et al. 2023). Specifically, expression data and treatment time points for *A. hypochondriacus* were retrieved from a previous transcriptome study, which profiled gene expression across various tissues and under drought conditions (Clouse et al. 2016). For *A. palmeri* and *A. tuberculatus*, time-course expression profiles in response to glufosinate and mesotrione herbicide treatments, respectively, were obtained, providing processed gene expression data linked to specific herbicide exposure durations (Salas-Perez et al. 2018; Kohlhase et al. 2019). Expression levels of *CO* genes were calculated using FPKM or RPKM metrics for each dataset to ensure accurate normalisation of transcript abundance across different conditions. The heatmaps were generated using R scripts. All figures were created using R software and Adobe Illustrato;r.

RESULTS AND DISCUSSION

Genome-wide identification of the CO transcription factor family in the Amaranthaceae family

To identify the CO gene family in the genomes of *Amaranthus* species, we conducted comprehensive database searches using CO protein sequences from *A. thaliana* (Griffiths et al. 2003). After filtering with HMMER (Potter et al. 2018), all putative members of the CO gene family in the reference genomes of *A. hypochondriacus*, *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor* were collected. As expected, a total of thirteen, thirteen, thirteen, twelve, twelve, and twelve members of the CO transcription factor family were found in *A. hypochondriacus*, *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor*, respectively. Interestingly, we observed that the number of *CO* genes varied slightly among the six *Amaranthus* species, with some having thirteen members and others twelve. This variation may be attributed to species-specific gene duplication or loss events, differences in genome assembly completeness, or annotation quality across species. Such discrepancies are commonly reported in comparative genomic studies and indicate natural evolutionary divergence within the same plant family (Lespinet et al. 2002). These annotations of the CO transcription factor family in six Amaranthaceae species are presented in Tables 1 and 2.

All members of the CO gene family in *A. hypochondriacus*, *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor* were located and designated based on their physical position on chromosome as *AhypCOL01* to *AhypCOL13*, *ApalCOL01* to *ApalCOL13*, *AcruCOL01* to *AcruCOL13*, *AhybCOL01* to *AhybCOL12*, *AtubCOL01* to *AtubCOL12*, and *AtrCOL01* to *AtrCOL12*, respectively (Tables 1 and 2). Of particular interest, thirteen members of the CO gene family in *A. hypochondriacus* were identified and mapped across sixteen chromosomes (Figure 1). Specifically, *AhypCOL01* and *AhypCOL02* were located on chromosome 1, *AhypCOL03* and *AhypCOL04* were found on chromosome 2, and *AhypCOL07* and *AhypCOL08* resided on chromosome 9. Five *CO* genes, including *AhypCOL05*, *AhypCOL06*, *AhypCOL09*, *AhypCOL10*, and

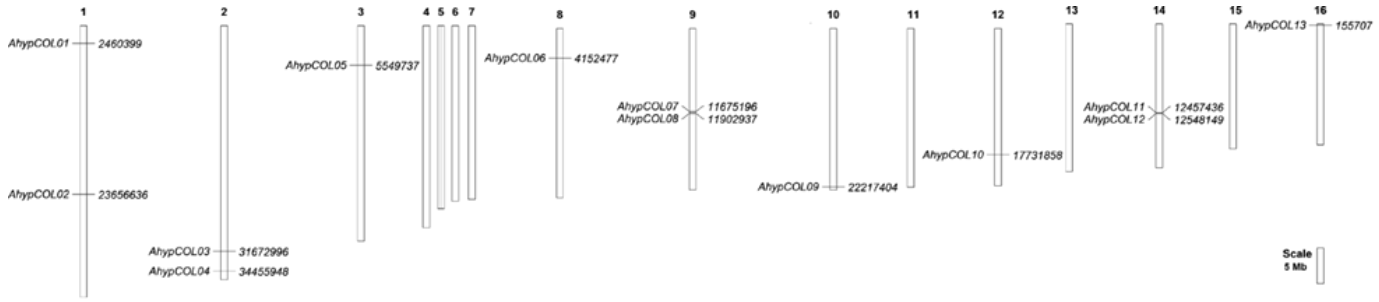

Figure 1. Physical localization of thirteen members of the CO gene family in *Amaranthus hypochondriacus*.

Table 1. Summary of the CO transcription factor family in *Amaranthus hypochondriacus*.

Gene name	Locus name	Genomic full -length (bp)	Protein full length (aa)	Molecular weight (kDa)	pI	GRAVY	Aliphatic index
AhypCOL01	AH000260	5334	410	44.84	5.26	-0.49	65.66
AhypCOL02	AH001280	6951	406	44.66	5.16	-0.57	62.73
AhypCOL03	AH004287	4715	318	35.73	4.35	-0.61	70.47
AhypCOL04	AH004578	4746	495	55.03	6.22	-0.53	75.86
AhypCOL05	AH005226	2857	426	41.10	5.80	-0.65	66.81
AhypCOL06	AH012867	2783	383	43.17	5.45	-0.65	69.48
AhypCOL07	AH014367	2774	392	44.96	5.28	-0.81	66.12
AhypCOL08	AH014379	1214	375	41.08	5.39	-0.34	72.32
AhypCOL09	AH016613	2005	370	41.07	5.46	-0.58	57.76
AhypCOL10	AH019184	1239	363	39.69	5.73	-0.52	57.93
AhypCOL11	AH020994	1228	377	41.89	5.52	-0.41	70.40
AhypCOL12	AH020998	2958	378	43.36	5.15	-0.95	59.02
AhypCOL13	AH022740	858	284	32.18	5.41	-0.67	62.89

AhypCOL13, were situated on chromosomes 3, 8, 10, and 16, respectively. Chromosome 14 also harboured two *CO* genes, namely *AhypCOL11* and *AhypCOL12*.

Previously, the number of members of CO gene families has been reported in various crop species. In *A. thaliana*, sixteen CO family genes have been identified and annotated (Griffiths et al. 2003). Similarly, the isolation of sixteen *CO* genes from rice has been reported (Griffiths et al. 2003). In the case of Chinese white pear, fifteen *CO* genes have been identified in the recent assembly (Wang et al. 2017). At least eleven *CO* genes have been reported in the *C. lavandulifolium* genome (Fu et al. 2015). Furthermore, nineteen and thirty-one candidate homologous *CO* genes have been identified in ginkgo (Yan et al. 2017) and mango (Liu et al. 2022), respectively. This variation in the number of *CO* genes among different species highlights the complexity and evolutionary dynamics of the CO gene family, suggesting that the expansion or contraction of these genes may play a crucial role in regulating flowering time and adaptation to diverse environmental conditions across plant taxa.

Determination of physicochemical properties of the CO transcription factor family in the Amaranthaceae family

To provide insights into the structural stability, solubility, and overall behaviour of the proteins under physiological conditions, we analysed the protein sequences of the CO transcription factor family in six *Amaranthus* species. As a result, the physicochemical features of the CO transcription factor families in the Amaranthaceae were described in Tables 1 and 2.

Firstly, we focused on the general parameters of the CO transcription factor in *A. hypochondriacus*. As shown in Table 1, the physicochemical analysis of the AhypCOL proteins in *A. hypochondriacus* revealed notable variations in their structural properties. The protein lengths ranged from 284 (AhypCOL13) to 495 amino acids (AhypCOL04), highlighting a diversity in

Table 2. Summary of the CO transcription factor family in other *Amaranthus* species.

Gene name	Locus name	Protein full length (aa)	Molecular weight (kDa)	pI	GRAVY	Aliphatic index
ApalCOL01	-	354	38.29	4.55	-0.43	64.80
ApalCOL02	Apalm008716.1	299	33.38	4.13	-0.52	73.01
ApalCOL03	Apalm008959.1	488	54.33	6.16	-0.59	73.93
ApalCOL04	Apalm009074.1	481	53.61	6.49	-0.60	71.56
ApalCOL05	Apalm011240.1	429	48.18	5.63	-0.56	68.62
ApalCOL06	Apalm027054.1	376	42.19	5.29	-0.66	68.96
ApalCOL07	Apalm031030.1	394	45.05	5.29	-0.81	69.26
ApalCOL08	Apalm031052.1	376	41.06	5.38	-0.38	71.62
ApalCOL09	Apalm034219.1	371	41.24	5.44	-0.59	57.87
ApalCOL10	Apalm039160.1	362	39.64	5.73	-0.55	57.27
ApalCOL11	Apalm043787.1	377	41.86	5.11	-0.40	71.67
ApalCOL12	Apalm043796.1	384	44.18	5.16	-0.97	59.11
ApalCOL13	Apalm048372.1	295	33.65	5.36	-0.76	54.24
AcruCOL01	Acrue000328.1	410	44.84	5.26	-0.50	65.66
AcruCOL02	-	354	38.34	4.53	-0.44	66.16
AcruCOL03	Acrue005567.1	484	53.65	5.85	-0.55	74.36
AcruCOL04	Acrue005876.1	299	33.43	4.08	-0.52	73.65
AcruCOL05	Acrue010732.1	426	48.06	5.74	-0.66	66.81
AcruCOL06	Acrue024490.1	383	43.13	5.52	-0.65	69.74
AcruCOL07	Acrue025858.1	379	41.38	5.39	-0.34	72.35
AcruCOL08	Acrue025882.1	392	44.96	5.33	-0.82	66.12
AcruCOL09	Acrue029840.1	371	41.27	5.44	-0.59	57.60
AcruCOL10	Acrue034394.1	362	39.60	5.73	-0.53	58.09
AcruCOL11	Acrue038814.1	377	41.81	5.63	-0.41	70.40
AcruCOL12	Acrue038825.1	378	43.31	5.06	-0.94	60.05
AcruCOL13	Acrue043364.1	285	32.34	5.41	-0.67	62.67
AhybCOL01	Ahybri000228.1	410	44.84	5.26	-0.50	65.66
AhybCOL02	-	354	38.34	4.53	-0.44	66.16
AhybCOL03	Ahybri004189.1	318	35.73	4.35	-0.61	70.47
AhybCOL04	Ahybri004868.1	426	48.12	5.70	-0.65	67.72
AhybCOL05	Ahybri012553.1	383	43.17	5.45	-0.66	69.48
AhybCOL06	Ahybri014249.1	391	44.81	5.39	-0.81	66.55
AhybCOL07	Ahybrind2_09	377	41.48	5.61	-0.33	74.01
AhybCOL08	Ahybri016450.1	371	41.27	5.44	-0.59	57.60
AhybCOL09	Ahybri018927.1	362	39.60	5.73	-0.53	58.09
AhybCOL10	Ahybri021005.1	377	41.89	5.52	-0.41	70.40
AhybCOL11	Ahybri021008.1	378	43.43	5.15	-0.95	60.05
AhybCOL12	Ahybri022681.1	285	32.34	5.41	-0.68	62.67
AtubCOL01	Atube000282.1	410	44.90	5.42	-0.50	66.85
AtubCOL02	Atube001848.1	401	44.20	5.12	-0.59	63.04
AtubCOL03	Atube005520.1	318	35.81	4.38	-0.64	66.51
AtubCOL04	Atube005822.1	439	49.31	5.90	-0.60	74.19
AtubCOL05	Atube006401.1	426	47.91	5.84	-0.59	67.49
AtubCOL06	Atube018650.1	391	44.63	5.50	-0.76	70.03
AtubCOL07	Atube018664.1	376	41.08	5.66	-0.37	72.39
AtubCOL08	Atube021699.1	370	41.07	5.38	-0.58	57.76
AtubCOL09	Atube024664.1	362	39.69	5.72	-0.55	57.54
AtubCOL10	Atube027408.1	377	41.80	5.40	-0.42	69.05
AtubCOL11	Atube027414.1	387	44.26	5.18	-0.88	60.18
AtubCOL12	-	297	33.59	5.95	-0.69	61.75
AtrCOL01	LOC130806235	410	45.03	5.47	-0.45	70.41
AtrCOL02	LOC130805640	406	44.74	5.13	-0.57	62.76
AtrCOL03	LOC130820990	325	36.65	4.58	-0.69	67.45
AtrCOL04	LOC130820595	484	54.02	6.03	-0.59	73.55
AtrCOL05	LOC130808794	429	48.23	5.55	-0.56	72.26
AtrCOL06	LOC130812668	384	43.32	5.53	-0.74	65.49
AtrCOL07	LOC130814712	393	45.15	5.29	-0.81	64.94

Table 2. Contd.

Gene name	Locus name	Protein full length (aa)	Molecular weight (kDa)	pI	GRAVY	Aliphatic index
AtrCOL08	LOC130814700	376	40.89	5.37	-0.34	71.62
AtrCOL09	LOC130804412	370	41.08	5.38	-0.58	57.76
AtrCOL10	LOC130803863	360	39.42	5.66	-0.56	55.69
AtrCOL11	LOC130824516	377	41.91	5.33	-0.43	66.50
AtrCOL12	LOC130824521	377	43.31	4.14	-0.90	58.17

Note: -: No information. Blank entries under the "Locus name" column indicate cases where locus information was not available in the corresponding genome annotations or databases at the time of analysis. The authors thank the reviewer for pointing this out. The blank rows in Table 2 indicate cases where locus information was not available in the reference genome or annotation databases at the time of analysis. The authors have rechecked the datasets to confirm this and have added a note in the table caption for clarification.

size that may correlate with functional specialisations among different CO family members. Correspondingly, the molecular weights of these AhypCOL proteins varied between 32.18 (AhypCOL13) and 55.03 kDa (AhypCOL04), which is consistent with the differences observed in their amino acid sequence lengths. The theoretical isoelectric points of the CO transcription factors were calculated to range from 4.35 (AhypCOL03) to 6.22 (AhypCOL04), indicating a spectrum of net charge distributions at physiological pH. Analysis of the aliphatic index showed values ranging from 57.76 (AhypCOL09) to 75.86 (AhypCOL04). The aliphatic index is a positive factor for the thermostability of globular proteins; thus, higher values imply greater stability across a range of temperatures. The grand average of hydropathicity values was found to be between -0.34 (AhypCOL08) and -0.95 (AhypCOL12). A negative grand average of hydropathicity values of the AhypCOL transcription factors in *A. hypochondriacus* indicated that these proteins were generally hydrophilic as previously described (Kyte & Doolittle 1982), which aligns with their expected localisation in the aqueous cellular environment and their involvement in protein-DNA and protein-protein interactions essential for transcriptional regulation.

Next, the physicochemical analysis of the CO transcription factor families in the five remaining *Amaranthus* species, including *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor*, also exhibited remarkable property variations. The protein lengths of the CO transcription factors ranged from 295 (ApalCOL13) to 488 (ApalCOL03), 285 (AcruCOL13) to 484 (AcruCOL03), 285 (AhybCOL12) to 426 (AhybCOL04), 297 (AtubCOL12) to 439 (AtubCOL04), and 325 (AtrCOL03) to 484 (AtrCOL04) amino acids across the *Amaranthus* species, including *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor*, respectively. Correspondingly, the molecular weights of these CO proteins varied between 33.38 (ApalCOL02) and 54.33 (ApalCOL03) kDa in *A. palmeri*, 32.34 (AcruCOL13) and 53.65 (AcruCOL03) kDa in *A. cruentus*, 32.34 (AhybCOL12) and 48.12 (AhybCOL04) kDa in *A. hybridus*, 33.59 (AtubCOL12) and 49.31 (AtubCOL04) kDa in *A. tuberculatus*, and 36.65 (AtrCOL03) and 54.02 (AtrCOL04) kDa in *A. tricolor*. Interestingly, the theoretical isoelectric points of all CO transcription factors in the five remaining *Amaranthus* species were less than 7.0 (acidic), ranging from 4.13 (ApalCOL02) to 6.49 (ApalCOL04) in *A. palmeri*, 4.08 (AcruCOL04) to 5.85 (AcruCOL03) in *A. cruentus*, 4.35 (AhybCOL03) to 5.73 (AhybCOL09) in *A. hybridus*, 4.38 (AtubCOL03) to 5.95 (AtubCOL12) in *A. tuberculatus*, and 4.14 (AtrCOL12) to 6.03 (AtrCOL04) in *A. tricolor*. The grand average of hydropathicity values of all CO transcription factors in *A. palmeri*, *A. cruentus*, *A. hybridus*, *A. tuberculatus*, and *A. tricolor* was found to be negative. This finding indicates that these proteins are generally hydrophilic. Additionally, analysis of the aliphatic index revealed values ranging from 54.24 to 73.93 in the ApalCOL transcription factor family in *A. palmeri*, 57.60 to 74.36 in the

AcruCOL transcription factor family in *A. cruentus*, 57.60 to 74.01 in the AhybCOL transcription factor family in *A. hybridus*, 57.54 to 74.19 in the AtubCOL transcription factor family in *A. tuberculatus*, and 55.69 to 73.55 in the AtrCOL transcription factor family in *A. tricolor*. The aliphatic index reflects the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine) and is considered a positive indicator of protein thermostability. Proteins with higher aliphatic index values are generally more stable at elevated temperatures and under extreme environmental conditions (Ikai 1980). This suggests that CO transcription factors with higher aliphatic index values may maintain functional integrity under stress. Although there is no strict universal threshold, aliphatic index values above 70 are often associated with enhanced thermostability in plant proteins (Ikai 1980).

The characteristics of the CO transcription factor families in higher plant species have also been investigated previously. For example, the CO transcription factor family in mango exhibited sequence lengths ranging from 492 to 1,536 base pairs, resulting in proteins composed of 163 to 511 amino acids (Liu et al. 2022). These proteins have molecular weights between 18.11 and 56.63 kDa and predicted isoelectric points from 3.93 to 8.85 (Liu et al. 2022). The predicted open reading frames of the *CO* genes in the grapevine ranged from approximately 1,044 bp for VviCOL4 to 1,425 bp for VviCOL14b (Wang et al. 2019). The corresponding polypeptides varied in length from 347 to 474 amino acids, resulting in calculated molecular masses between approximately 38.00 and 51.43 kDa (Wang et al. 2019). In the case of *C. lavandulifolium*, the CO transcription factor family sizes have been reported to range from 337 to 434 amino acid residues (Fu et al. 2015). Additionally, the protein size of the CO transcription factor family in Chinese white pear ranged from 340 and 488 amino acid residues (Wang et al. 2017), and from 253 to 575 amino acid residues in quinoa (Tran et al. 2025). In summary, the physicochemical properties of the CO transcription factors, including protein length, molecular weight, isoelectric point, aliphatic index, and grand average of hydropathicity values, provide valuable insights into their structural stability and functional potential. These parameters lay the groundwork for further structural and functional analyses, contributing to a deeper understanding of how CO proteins regulate flowering time in response to photoperiodic signals.

Classification of the CO transcription factor family in the Amaranthaceae family

To assess the relationships of CO transcription factors in the Amaranthaceae family, an unrooted phylogenetic tree of the CO proteins from six *Amaranthus* species and well-characterised CO proteins from *A. thaliana* (Griffiths et al. 2003) and sugar beet (Chia et al. 2008) was successfully constructed. As a result, Figure 2 presents the Maximum Likelihood-based phylogenetic tree of all members of the CO transcription factor families in the Amaranthaceae family and relatives.

All members of the CO transcription factor families isolated from six *Amaranthus* species were categorised into three groups through phylogenetic analysis comparing CO proteins from *A. thaliana* (Griffiths et al. 2003) and sugar beet (Chia et al. 2008). Particularly, a total of twenty-nine members of the CO transcription factor families in the Amaranthaceae family were placed in Group I, with three members in each CO transcription factor family in six *Amaranthus* species, including AhybCOL07, AhybCOL09, AhybCOL10 from *A. hybridus*, AhypCOL08, AhypCOL10, AhypCOL11 from *A. hypochondriacus*, AcruCOL07, AcruCOL10, AcruCOL11 from *A. cruentus*, ApalCOL08, ApalCOL10, ApalCOL11 from *A. palmeri*, AtubCOL07, AtubCOL09, AtubCOL10 from *A. tuberculatus*, and AtriCOL08, AtriCOL10, AtriCOL11 from *A.*

tricolor, assigned to subgroup Ia. Meanwhile, eleven members of the CO transcription factor families in six *Amaranthus* species, particularly AhubCOL08, AhybCOL12 from *A. hybridus*, AhypCOL09, AhypCOL13 from *A. hypochondriacus*, AcruCOL09, AcruCOL13 from *A. cruentus*, ApalCOL09, ApalCOL13 from *A. palmeri*, AtubCOL08, AtubCOL12 from *A. tuberculatus*, and AtriCOL09 from *A. tricolor*, were positioned in subgroup Ib. Next, two members in each CO transcription factor family in six *Amaranthus* species, including AhybCOL06 and AhybCOL11, AhypCOL07 and AhypCOL12, AcruCOL08 and AcruCOL12, ApalCOL07 and ApalCOL12, AtubCOL06 and AtubCOL11, and AtriCOL07 and AtriCOL12 from *A. hybridus*, *A. hypochondriacus*, *A. cruentus*, *A. palmeri*, *A. tuberculatus*, and *A. tricolor*, respectively, were grouped in group II. Finally, the remaining thirty-four members of the CO transcription factor families in *A. hybridus*, *A. hypochondriacus*, *A. cruentus*, *A. palmeri*, *A. tuberculatus*, and *A. tricolor* were classified under group III.

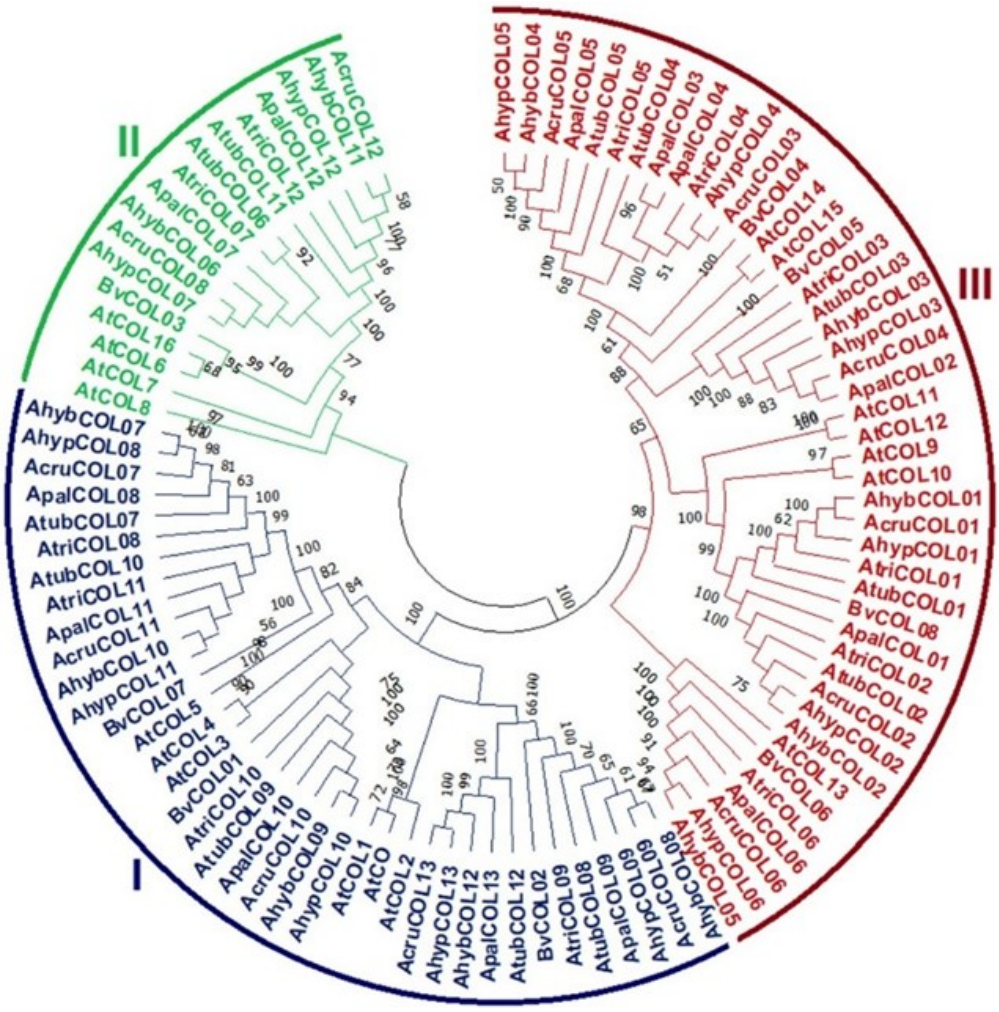

Figure 2. Categorization of the CO transcription factor families in the Amaranthaceae family.

Previously, the classification of the CO transcription factor families in higher plant species was reported. The twelve *CO* genes isolated from grapevine were categorized into three groups through phylogenetic analysis (Wang et al. 2019). For example, three members, including VviCOL2, VviCOL4, and VviCOL5, were placed in group I, while VviCOL16a and VviCOL16b were classified under group II, and VviCOL9a, VviCOL9b, VviCOL11a, VviCOL11b, VviCOL13, VviCOL14a, and VviCOL14b were grouped in group III (Wang et al. 2019). Furthermore, to investigate the evolutionary relationships among CO proteins, a phylogenetic tree was constructed using sixty members of the CO transcription factor families from *A.*

thaliana, grapevine, and mango (Liu et al. 2022). As expected, these CO proteins were clearly divided into three distinct clades, which largely reflected their structural differences (Liu et al. 2022). This classification has been reported to be similar to that of the CO transcription factor families in other crop species, such as barley (Griffiths et al. 2003), rice (Griffiths et al. 2003), Chinese white pear (Wang et al. 2017), *C. lavandulifolium* (Fu et al. 2015), ginkgo (Yan et al. 2017), and quinoa (Tran et al. 2025).

Gene structure analysis of the CO transcription factor family in *Amaranthus hypochondriacus*

The gene structure of the CO transcription factor family in *A. hypochondriacus* was analysed to investigate the exon-intron organization and potential structural variations among the gene family members. Using the GSDS tool (Hu et al. 2015), full-length genomic sequences were aligned with their corresponding coding sequences to identify the arrangement of exons and introns. The results revealed significant variation in the number of exons among different *CO* genes in *A. hypochondriacus* (Figure 3)

The gene structure analysis showed that some *AhypCOL* genes possessed multiple exons. In contrast, only one member of the CO gene family, namely *AhypCOL13*, had no introns, reflecting the evolutionary divergence within the CO gene family in *A. hypochondriacus*. This could be a genuine biological feature, as intronless genes are commonly found in plant genomes and are often associated with gene duplication events, such as retrotransposition or intron loss through evolution (Chen et al. 2023). For instance, *CO* genes in closely related clades of the phylogenetic tree exhibited similar exon-intron patterns, indicating possible conservation of gene structure in evolutionarily related CO family members in *A. hypochondriacus*. Conversely, variations in exon-intron distribution in more distantly related genes could indicate functional diversification across different CO family members in *A. hypochondriacus*. In particular, the number of exons in the *CO* genes in *A. hypochondriacus* ranged from one (*AhypCOL13*) to five (*AhypCOL04*). Among them, a large number of the *CO* genes in *A. hypochondriacus*, particularly six out of thirteen members, contained two exons, while five (out of thirteen) members of the *CO* genes in *A. hypochondriacus* had four exons. These findings highlight the structural diversity within the CO transcription factor family in *A. hypochondriacus*, which may contribute to the regulation of photoperiodic flowering in response to environmental cues.

Recently, the genomic organisation of the thirty *CO* genes in mango has shown significant variation (Liu et al. 2022). Specifically, three *MiCOL* genes had four introns, eight contained three introns, nine had two introns, ten possessed only one intron, and *MiCOL11* was entirely devoid of introns (Liu et al. 2022). Regarding the *CO* genes in grapevine, *VviCOL9a*, *VviCOL11a*, *Vvi-*

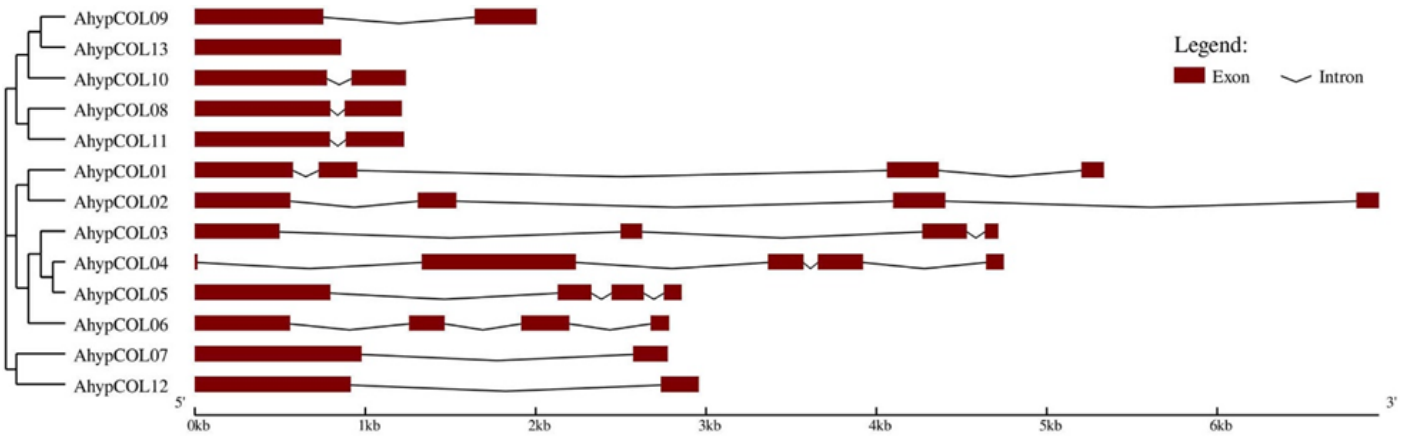

Figure 3. Determination of gene structure of the CO transcription factor family in *Amaranthus hypochondriacus*.

COL11b, *VviCOL13*, *VviCOL14a*, and *VviCOL14b* each possessed three introns. In contrast, *VviCOL2*, *VviCOL4*, *VviCOL5*, *VviCOL16a*, and *VviCOL16b* were found to contain only one intron (Wang et al. 2019). Taken together, these structural differences highlight the diversity within the CO gene family in *A. hypochondriacus* and possibly in other crop species.

Expression patterns analysis of the CO transcription factor family in the Amaranthaceae family

As a main part of this study, we investigated the expression patterns of the CO gene family in several species belonging to the Amaranthaceae family. As a result, four RNA-Seq datasets from *A. hypochondriacus*, *A. palmeri*, and *A. tuberculatus* were analysed to assess the expression patterns of the CO gene family in major tissues under various conditions. Figures 4 and 5 described the expression patterns of the CO gene family in *A. hypochondriacus*, *A. palmeri*, and *A. tuberculatus*.

In *A. hypochondriacus*, the expression patterns of the *AhypCOL* gene family varied across major organs during the growth and development of plants. We found that three *CO* genes, including *AhypCOL09*, *AhypCOL10*, and *AhypCOL11*, were highly expressed in leaf tissues, while only *AhypCOL10* was exclusively expressed in stem and floral samples (Figure 4A). Two *CO* genes, *AhypCOL09* and *AhypCOL10*, were up-regulated in drought-treated tissues. Furthermore, it has been reported that *AhypCOL09* was mainly expressed in stem, root, flower, and mature seed samples, while *AhypCOL10* was exclusively expressed in the main organs, such as leaf, stem, root, flower, and mature seed (Figure 4B).

In *A. palmeri*, seven (out of thirteen) members of the CO gene family were detected in their expression levels in glufosinate-treated plants. In particular, three *CO* genes, including *ApalCOL03*, *ApalCOL07*, and *ApalCOL11*, were not altered in both glufosinate-sensitive and glufosinate-resistant plants under treatments (Figure 5A). Two *CO* genes, including *ApalCOL09* and *ApalCOL10*, were induced in glufosinate-sensitive and/or glufosinate-resistant plants under treatments. *ApalCOL13* expression decreased in glufosinate-resistant plants (~ -2.12-fold), whereas *ApalCOL04* showed contrasting expression levels in glufosinate-sensitive and glufosinate-resistant plants by ~ -1.98-fold and 2.41-fold, respectively. In *A. tuberculatus*, most *CO* genes (eleven out of twelve) exhibited their interesting expression patterns in leaves at 3, 6, 12, and 24 hours after mesotrione treatment (Figure 5B). For example, four *CO* genes, including *AtubCOL01*, *AtubCOL02*, *AtubCOL03*, and

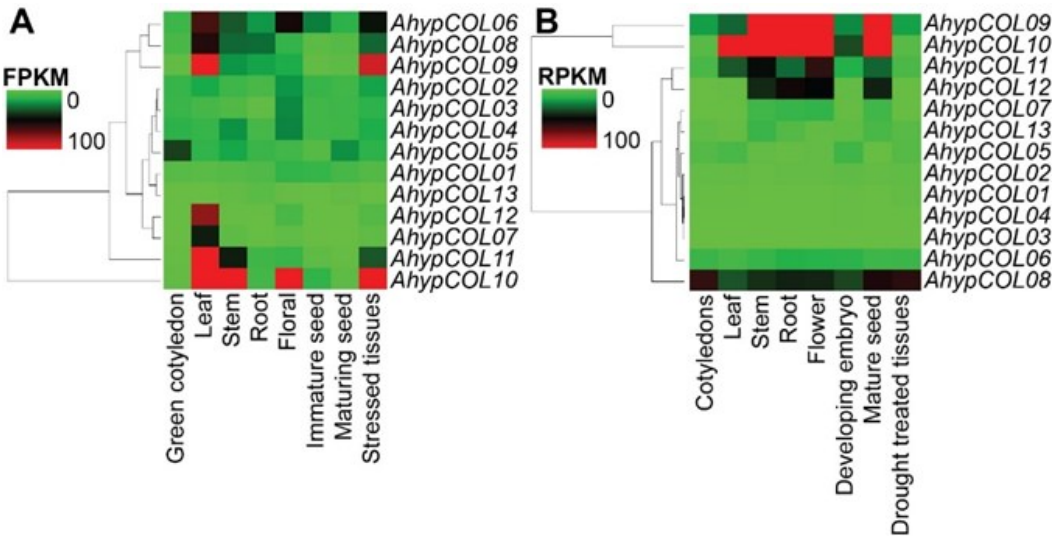

Figure 4. Expression patterns of the CO gene family in *Amaranthus hypochondriacus* based on (A) Phytozome and (B) AGRDB databases.

AtubCOL04, were down-regulated in leaves after three hours of treatment but were up-regulated in these examined tissues after 6 and/or 12 and/or 24 hours of treatment. Interestingly, *AtubCOL05* and two other *CO* genes, *AtubCOL08* and *AtubCOL09*, were induced and reduced in leaves under the treatments, respectively. Meanwhile, three *CO* genes, including *AtubCOL06*, *AtubCOL10*, and *AtubCOL11*, were up-regulated in leaves after 3 and/or 6 hours of treatment but down-regulated in these tissues after 12 and/or 24 hours. Taken together, our reanalysis of RNA-Seq datasets revealed that the CO gene family in three *Amaranthus* species was differentially expressed in various organs during the growth and development.

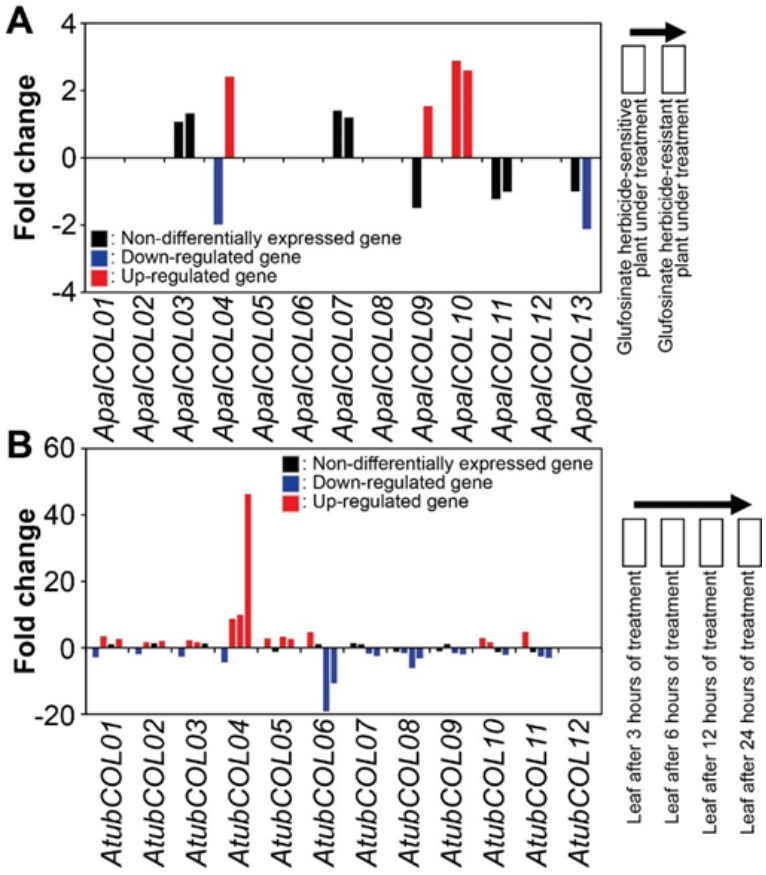

Figure 5. Expression patterns of the CO gene families in (A) *Amaranthus palmeri* and (B) *A. tuberculatus*.

CONCLUSION

In conclusion, the comprehensive genome-wide identification, structural analysis, and expression profiling of the CONSTANS (CO) transcription factor family in the Amaranthaceae family have provided significant insights into the genetic and functional diversity of these genes. The identification of twelve to thirteen *CO* genes across six *Amaranthus* species revealed their chromosomal localisation and structural diversity, with varying exon-intron arrangements suggesting functional diversification. Physicochemical analyses further highlighted structural differences, including variations in protein length, molecular weight, and isoelectric points. Phylogenetic analysis classified the CO transcription factor family into three major groups, aligning with known CO proteins from *A. thaliana* and sugar beet, thereby offering a framework for understanding evolutionary relationships within the CO gene family. Gene expression analysis across multiple RNA-Seq datasets demonstrated tissue-specific and stress-responsive regulation of *CO* genes, with certain members highly expressed under environmental stresses such as drought and herbicide treatments. This study could enhance our understanding of the role of CO transcription factors in regulating photoperiodic flowering and stress

responses in *Amaranthus* species, thereby contributing to future agricultural applications and crop improvement strategies.

AUTHOR CONTRIBUTION

X.D.V. designed the research, collected and analysed data, and prepared the first manuscript draft. P.B.C. co-designed the research, collected and analysed data, edited the manuscript, and supervised the study. Q.P.N., T.T.T.H., D.D.N., and Q.T.N.L. contributed to data collection, with T.T.T.H., D.D.N., and Q.T.N.L. also involved in data analysis. H.V.L. contributed to research design, data collection, and analysis. H.D.C. co-designed the research, collected and analysed data, edited the manuscript, and supervised the study

ACKNOWLEDGMENTS

This work was funded by the fundamental research programme of Hung Vuong University under project grant No. 23/2024/KHCN (HV23.2024).

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the research or funding.

REFERENCES

Baguma, J.K. et al., 2023. Flowering and fruit-set in cassava under extended red-light photoperiod supplemented with plant-growth regulators and pruning. *BMC Plant Biology*, 23(1), 335. doi: 10.1186/s12870-023-04349-x

Baraniak, J. & Kania-Dobrowolska, M., 2022. The dual nature of amaranth-functional food and potential medicine. *Foods*, 11(4), 618. doi: 10.3390/foods11040618

Cao, P.B., 2022. *In silico* structural, evolutionary, and expression analysis of small heat shock protein (shsp) encoding genes in cocoa (*Theobroma cacao* L.). *Journal of Animal and Plant Sciences*, 32(5), pp.1394-1402. doi:10.36899/JAPS.2022.5.0546

Caselato-Sousa, V.M. & Amaya-Farfán, J., 2012. State of knowledge on amaranth grain: a comprehensive review. *Journal of Food Science*, 77(4), pp.R93-104. doi: 10.1111/j.1750-3841.2012.02645.x

Chen, Y. et al., 2023. Trends in the evolution of intronless genes in Poaceae. *Frontiers in Plant Science*, 14, 1065631. doi: 10.3389/fpls.2023.1065631

Chia, T.Y. et al., 2008. Sugar beet contains a large CONSTANS-LIKE gene family including a CO homologue that is independent of the early-bolting (B) gene locus. *Journal of Experimental Botany*, 59(10), pp.2735-2748. doi: 10.1093/jxb/ern129

Chu, H.D. et al., 2018. Identification, structural characterization and gene expression analysis of members of the Nuclear Factor-Y family in chickpea (*Cicer arietinum* L.) under dehydration and abscisic acid treatments. *International Journal of Molecular Sciences*, 19(11), 3290. doi: 10.3390/ijms19113290

Chu, H.D. et al., 2024. Identification of Two Enzymes for Trehalose Synthesis and Their Potential Function in Growth and Development in Peanut (*Arachis hypogaea*). *Journal of Tropical Life Science*, 14(1), pp.83-94. doi: 10.11594/jtls.14.01.10

Chu, N.T.B. et al., 2024. Genome-wide identification, characterization, and expression analysis of the small auxin-up RNA gene family during zygotic and somatic embryo maturation of the cacao tree (*Theobroma cacao*). *Genomics & Informatics*, 22(1), 2. doi: 10.1186/s44342-024-00003-6

Clouse, J.W. et al., 2016. The Amaranth Genome: Genome, Transcriptome, and Physical Map Assembly. *Plant Genome*, 9(1), plantgenome2015.07.0062. doi: 10.3835/plantgenome2015.07.0062

Dahal, P. et al., 2022. Crystal structure of a tandem B-box domain from Arabidopsis CONSTANS. *Biochemical and Biophysical Research Communications*, 599, pp.38-42. doi: 10.1016/j.bbrc.2022.02.025

Fu, J. et al., 2015. Identification and characterization of the CONSTANS-like gene family in the short-day plant Chrysanthemum lavandulifolium. *Molecular Genetics and Genomics*, 290(3), pp.1039-1054. doi: 10.1007/s00438-014-0977-3

Gasteiger, E. et al., 2003. ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research*, 31(13), pp.3784-3788. doi: 10.1093/nar/gkg563

Gasteiger, E. et al., 2005. Protein identification and analysis tools on the ExPASy server. In *The proteomics protocols handbook*. Humana Press, pp.571-607. doi: 10.1385/1-59259-890-0:571

Goodstein, D.M. et al., 2012. Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Research*, 40(Database issue), pp.D1178-D1186. doi: 10.1093/nar/gkr944

Griffiths, S. et al., 2003. The evolution of CONSTANS-like gene families in barley, rice, and Arabidopsis. *Plant Physiology*, 131(4), pp.1855-1867. doi: 10.1104/pp.102.016188

Hu, B. et al., 2015. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics*, 31(8), pp.1296-1297. doi: 10.1093/bioinformatics/btu817

Ikai, A., 1980. Thermostability and Aliphatic Index of Globular Proteins. *The Journal of Biochemistry*, 88(6), pp.1895-1898. doi: 10.1093/oxfordjournals.jbchem.a133168

Jan, N. et al., 2023. Amaranth and quinoa as potential nutraceuticals: A review of anti-nutritional factors, health benefits and their applications in food, medicinal and cosmetic sectors. *Food Chemistry X*, 18, 100687. doi: 10.1016/j.fochx.2023.100687

Kim, S.Y. et al., 2008. Regulation of CONSTANS and FLOWERING LOCUS T expression in response to changing light quality. *Plant Physiology*, 148(1), pp.269-279. doi: 10.1104/pp.108.122606

Kohlhase, D.R. et al., 2019. Using RNA-seq to characterize responses to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor herbicide resistance in waterhemp (*Amaranthus tuberculatus*). *BMC Plant Biology*, 19 (1), 182. doi:10.1186/s12870-019-1795-x

Kumar, S. et al., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology Evolution*, 35(6), pp.1547-1549. doi: 10.1093/molbev/msy096

Kyte, J. & Doolittle, R.F., 1982. A simple method for displaying the hydropathic character of a protein. *Journal of Molecular Biology*, 157(1), pp.105-132. doi: 10.1016/0022-2836(82)90515-0

La, H.V. et al., 2022. Insights into the gene and protein structures of the CaSWEET family members in chickpea (*Cicer arietinum*), and their gene expression patterns in different organs under various stress and abscisic acid treatments. *Gene*, 819, pp.146210. doi: 10.1016/j.gene.2022.146210

Larkin, M.A. et al., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23(21), pp.2947-2948. doi: 10.1093/bioinformatics/btm404

Lespinet, O. et al., 2002. The role of lineage-specific gene family expansion in the evolution of eukaryotes. *Genome Research*, 12(7), pp.1048-1059. doi: 10.1101/gr.174302

Liu, Y. et al., 2022. Genome-wide identification of the mango CONSTANS (CO) family and functional analysis of two MiCOL9 genes in transgenic *Arabidopsis*. *Frontiers in Plant Science*, 13, 1028987. doi:10.3389/fpls.2022.1028987

Ma, X. et al., 2021. A chromosome-level *Amaranthus cruentus* genome assembly highlights gene family evolution and biosynthetic gene clusters that may underpin the nutritional value of this traditional crop. *Plant Journal*, 107(2), pp.613-628. doi: 10.1111/tpj.15298

Mistry, J. et al., 2021. Pfam: The protein families database in 2021. *Nucleic Acids Research*, 49(D1), pp.D412-D419. doi: 10.1093/nar/gkaa913

Montgomery, J.S. et al., 2020. Draft Genomes of *Amaranthus tuberculatus*, *Amaranthus hybridus*, and *Amaranthus palmeri*. *Genome Biology and Evolution*, 12(11), pp.1988-1993. doi: 10.1093/gbe/evaa177

Potter, S.C. et al., 2018. HMMER web server: 2018 update. *Nucleic Acids Research*, 46(W1), pp.W200-W204. doi: 10.1093/nar/gky448

Pulvento, C. & Sellami, M.H., 2021. Yield and quality of *Amaranthus hypochondriacus* grain amaranth under drought and salinity at various phenological stages in southern Italy. *Journal of the Science of Food and Agriculture*, 102, pp.5022-5033. doi: 10.1002/jsfa.11088

Roeber, V.M. et al., 2021a. Light acts as a stressor and influences abiotic and biotic stress responses in plants. *Plant, cell & environment*, 44(3), pp.645-664. doi:10.1111/pce.13948

Roeber, V.M. et al., 2021b. The Photoperiod: Handling and causing stress in plants. *Frontiers in Plant Science*, 12, 781988. doi:10.3389/fpls.2021.781988

Salas-Perez, R.A. et al., 2018. RNA-Seq transcriptome analysis of Amaranthus palmeri with differential tolerance to glufosinate herbicide. *PLoS One*, 13(4), e0195488. doi:10.1371/journal.pone.0195488

Singh, A. et al., 2023. Amaranth Genomic Resource Database: an integrated database resource of Amaranth genes and genomics. *Frontiers in Plant Science*, 14, 1203855. doi:10.3389/fpls.2023.1203855

Soriano-García, M. & Aguirre-Díaz, I.S., 2019. Nutritional functional value and therapeutic utilization of Amaranth. In *Nutritional value of amaranth*. IntechOpen.

Sunil, M. et al., 2014. The draft genome and transcriptome of *Amaranthus hypochondriacus*: a C4 dicot producing high-lysine edible pseudo-cereal. *DNA Research*, 21(6), pp.585-602. doi:10.1093/dnares/dsu021

Thompson, J.D. et al., 2002. Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc Bioinformatics*, Chapter 2, Unit 2.3. doi: 10.1002/0471250953.bi0203s00

Tran, H.T.T. et al., 2025. Genome-wide analysis of CONSTANS-like (CqCOL) transcription factors in quinoa (*Chenopodium quinoa*): Structural diversity, phylogeny, and stress-responsive expression. *HAYATI Journal of Biosciences*, 32(5), pp.1135-1146. doi: 10.4308/hjb.32.5.1135-1146

Wang, H. et al., 2023. Chromosome-scale *Amaranthus tricolor* genome provides insights into the evolution of the genus Amaranthus and the mechanism of betalain biosynthesis. *DNA Res*, 30(1), dsac050. doi: 10.1093/dnares/dsac050

Wang, L. et al., 2019. Genome-wide identification, phylogenetic analysis, and expression profiling of CONSTANS-like (COL) genes in *vitis vinifera*. *Journal of Plant Growth Regulation*, 38(2), pp.631-643. doi: 10.1007/s00344-018-9878-8

Wang, P. et al., 2017. PbCOL8 is a clock-regulated flowering time repressor in pear. *Tree Genetics & Genomes*, 13(5), 107. doi: 10.1007/s11295-017-1188-y

Wenkel, S. et al., 2006. CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of *Arabidopsis*. *Plant Cell*, 18(11), pp.2971-2984. doi:10.1105/tpc.106.043299

Yan, J. et al., 2017. Isolation and functional characterization of a circadian-regulated CONSTANS homolog (GbCO) from *Ginkgo biloba*. *Plant Cell Report*, 36(9), pp.1387-1399. doi: 10.1007/s00299-017-2162-8

Zhang, B. et al., 2023. Involvement of CONSTANS-like proteins in plant flowering and abiotic stress response. *International Journal of Molecular Sciences*, 24(23), 16585. doi: 10.3390/ijms242316585