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Comparative Transcriptomic Profiling of Auxin Response Factor Genes Reveals Tissue-Specific Expression Patterns in Brassica napus

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ABSTRACT
The family of genes known as Auxin Response Factor (ARF) are crucial for the growth and development of plants, as well as the hormone control of transcription. In this study, comparative transcriptomic analysis and functional annotation were used to examine tissue-specific expression patterns of ARF-related genes in *Brassica napus*. Descriptive statistical analysis, expression comparison and replicate correlation were conducted on the gene expression data obtained from root and leaf tissues. The data set consisted of 124 records of genes and 90 genes IDs in 15 experimental samples. The mean expression in root control samples (7.999) was higher than the mean expression in leaf control samples (4.573), reflecting variation in expression across tissues. Among several candidate genes there were some that showed striking expression levels in the roots, such as BnaA05G0014400ZS and BnaC04G0014300ZS. Replications of correlations were between 0.783 and 0.990 indicating a generally consistent expression profile across experimental groups. Of the 50 records annotated, 50 were annotated with Gene Ontology information and 20 were annotated with KEGG pathway annotations. The uncertainty of expression normalization and incomplete annotations, however, hindered a clear biological interpretation. These results offer initial ideas of transcriptional variation associated with ARF and lay the groundwork for future functional studies of auxin-related regulatory processes in *B. napus*.

Keywords: *Brassica napus*; Auxin Response Factor Genes; Comparative Transcriptomics; Tissue-Specific Gene Expression; Functional Annotation

1. Introduction

Rapeseed/canaola (*Brassica napus L.*) is an important global oilseed crop used for human consumption as edible oil, as animal feed and for industrial uses. It relies on intricate molecular regulatory processes to control cell differentiation, organ formation and environmental responses for its growth, productivity and adaptability. To enhance crop performance, and discover genes involved in developmental regulation, it's crucial that one understands the genes and molecular mechanisms that control these biological processes. Over the last years, transcriptomic technologies have evolved to allow a detailed analysis of gene expression at various developmental stages, tissues and environmental conditions. In B. napus, which is an allotetraploid species, spatiotemporal transcriptome profiling revealed significant differences in the expression of genes among tissues and subgenomes, emphasizing the tissue-specific regulation of transcription in this species (Wei et al., 2022). In addition, comparative transcriptomic studies have identified specific reproductive development and phytohormone metabolism-related regulatory patterns, highlighting the need for a multi-level approach by combining gene expression analysis with developmental physiology (Jiang et al., 2025).

An essential plant growth factor, auxin controls a wide range of plant processes, such as cell expansion, differentiation of tissues, vascular development, root architecture and organ initiation. The auxin mediated transcriptional response is mainly controlled by interactions between auxin receptors, Auxin/Inhibitor of Auxin (Aux/IAA) transcriptional repressors and Auxin Response Factor (ARF) proteins. ARFs act as transcription regulators that bind to auxin-responsive elements in target genes' promoters and regulate the expression of downstream genes. Both developmental and regulatory activities play a role in developmental plasticity, as they coordinate cellular reactions to endogenous hormonal signals and environmental factors. In *Brassica* species, transcriptomic studies have revealed links between auxin-related gene expression and specific developmental processes, such as hypocotyl enlargement and storage-organ formation (Huang et al., 2023). The observations made here indicate that analysis of ARF gene expression in different tissues could be useful for understanding the molecular events that are involved in tissue differentiation and auxin-mediated developmental control.

The polyploid genome of B. napus is an additional complication of its transcriptional regulation, because of the A and C subgenomes that it represents, which are of ancestral origin to *Brassica* species. Various families of transcription factors have been expanded and diversified through genome duplication, the retention of the duplicated genes, and the functional evolution of the duplicated genes. This means that duplicated genes can have different patterns of expression, different regulatory functions, and different signals to which they respond in the course of development and/or environmental conditions. Transcription factor network and subgenome-associated gene expression bias identified during seed development highlight the need to investigate the expression of regulatory genes within the genomic architecture of B. napus (Khan et al., 2022). Likewise, evolutionary relationships and expression patterns of KNOX transcription factors have been revealed by genome-wide characterization of their expression patterns, which revealed their association with developmental regulation (He et al., 2025). Findings will serve as a basis for studying the tissue-specific variation in the transcription of ARF-related genes.

Transcriptomic profiling is now an important tool in characterization of gene families and possible roles in regulation in plants. By using RNA sequencing and related bioinformatic methods, researchers are able to assess transcript abundance, look for differences in expression and explore the functional connections between genes. In previous studies of non-specific lipid transfer protein genes in rapeseed, high expression variation was found in response to biotic and abiotic stresses (Xue et al., 2022). Similarly, analyses of the expression patterns of two-component system genes revealed various stress responses in the transcription level, reflecting the fact that the expression patterns differ, depending on the function of the gene and the physiological context (Liu et al., 2023). Functional divergence and stress-associated expression traits have also been shown at the genome-wide level for regulatory gene families containing BZR transcription factors (Sarwar et al., 2022). These results collectively demonstrate the power of comparative expression profiling to identify candidate regulatory genes and to gain insight into transcriptional specialization.

The analysis of tissue-specific expression is especially significant because the roots and leaves have different physiological functions and react differently to the hormonal and environmental signals. The roots will mainly be involved in nutrient and water uptake, interact with the soil environment around them and provide anchorage, while leaves will be involved in the production and uptake of assimilates, photosynthesis and gas exchange. These functional differences are accompanied with distinct transcription requirements and regulatory pathways. Single-cell transcriptomic analysis of the leaf vasculature of *Brassica rapa* has revealed both developmental diversity and differing patterns of gene expression amongst specialized cellular populations (Guo et al., 2025). Likewise, studies on phosphorus stress adaptation have

identified tissue-specific molecular responses, such as metabolic remodeling in leaves and hormonal regulation in roots (Zulfiqar et al., 2025). These findings highlight the need to investigate the expression of genes in specific tissue contexts and not only use whole-plant transcriptional data.

Comparative transcriptomic studies have also been shown to be valuable in the study of developmental transitions and differences in organ regulation. The molecular changes which occurred during the early differentiation of tissues in kohlrabi and the initiation of the storage organ formation were found during transcriptome analysis in the kohlrabi stem during development (Nuruzzaman et al., 2022). To investigate the presence of organ-specific regulatory programs, temporal expression patterns of B. napus were analyzed in shoot apices and leaves during pre-floral development, showing opposing trends (Jones et al., 2024). Comparative analyses of reproductive organs have also shown conserved and divergent gene expression patterns within *brassica* species (Chen et al., 2025). In addition to development, integrated transcriptomic studies have revealed heat stress-induced gene expression coordination and regulatory networks in rapeseed (Shahsavari et al., 2025). Genome-wide characterization of SET domain family genes has also helped in understanding the diversity of regulatory proteins of *B. napus* (Sehrish et al., 2022).

Nevertheless, a specific analysis of the gene expression associated with ARF in roots and leaves is still useful to understand the potential role of the ARF in the regulation of tissue-specific auxin. In the current study, comparative transcriptomic profiling and functional annotation are used to describe the expression pattern of ARFs in *B. napus*. In this investigation, tissue-associated expression variation is examined, and candidate regulatory genes are identified, to serve as the basis for understanding the transcriptional organization of auxin responsive pathways as well as to inform further functional investigations. The objectives of this research are as follows:

- To characterize Auxin Response Factor gene expression profiles across root and leaf tissues of *Brassica napus.*
- To identify tissue-enriched ARF genes through comparative analysis of transcriptomic expression patterns across experimental samples.
- To investigate functional annotations and biological pathways associated with tissue-dependent ARF gene expression in *Brassica napus.*

2. Materials and Methods

2.1. Study Design and Transcriptomic Dataset Description

This was a comparative transcriptomics study using data to examine the expression of Auxin Response Factor (ARF) genes in *Brassica napus* (Purushotham, 2024). The gene expression data uploaded is a data set of measurements of different experimental conditions obtained from root and leaf samples. Fifteen expression columns were identified including 9 root and 6 leaf samples. Root samples were from CK, Z-12, and Z-4 groups while leaf samples were from CK and Z groups. There were three samples in each group. A tissue expression library of genes was analysed for both identification and functional annotation to define the structure of the library and its potential for tissue-specific expression profiling.

2.2. Data Preprocessing and ARF Gene Identification

The dataset was analyzed using data processing procedures based on Python to separate ARF-related gene records and structure the expression measurements. Gene identifiers, annotation fields and sample-specific expression values were checked for completeness and consistency. Multiple gene identifiers were found and the first one was kept for descriptive gene-level summary. Values were not artificially substituted for values missing from expression. Annotations of function were removed from numerical expression fields and samples were classified based on tissue and condition. Comparative analysis was then carried out, with the help of expression distributions.

2.3. Comparative Gene Expression Analysis

Comparative expression analysis was designed to be able to check the differences in the expression of ARF genes between root and leaf tissues. Expressions were summarized separately in each experimental group, and the best direct comparison of tissue level references were the control samples. Available observations were summed and divided to determine the mean expression value at each gene. Root-to-leaf expression ratios were explored to find candidate tissue-enriched genes. Conventional log2 fold change and differential expression significance testing was not used without first ensuring the correct nature of the expression-data format as the data set contained missing and/or negative values.

2.4. Expression Pattern Clustering and Multivariate Analysis

For expression pattern analysis, the structure was designed to analyze similarities and differences between ARF genes and experimental samples. A gene by sample matrix was created based on available numerical measurements. Exploratory methods used to determine genes with similar expression patterns were

hierarchical clustering, and principal component analysis was chosen for multivariate sample variation. Preprocessing constraints of missing values and uncertainty of measurements on the scales were acknowledged. Therefore, imputation, transformation and scaling procedures needed to be validated prior to obtaining meaningful sample separation in tissue and/or treatment groups through clustering and PCA.

2.5. Functional Annotation and Statistical Analysis

For expression pattern analysis, the structure was designed to analyze similarities and differences between ARF genes and experimental samples. A gene by sample matrix was created based on available numerical measurements. Exploratory methods used to determine genes with similar expression patterns were hierarchical clustering, and principal component analysis was chosen for multivariate sample variation. Preprocessing constraints of missing values and uncertainty of measurements on the scales were acknowledged. Therefore, imputation, transformation and scaling procedures needed to be validated prior to obtaining meaningful sample separation in tissue and/or treatment groups through clustering and PCA.

3. Results

3.1. Transcriptomic Dataset Characteristics and ARF Gene Distribution

The 124 gene identifier records (90 unique genes) in the transcriptomic data set were for *Brassica napus*. The measurements of the expressions were distributed in 15 samples, nine root and six leaf samples. Total 5 experimental groups with 3 samples each were identified. There were repeated gene identifiers in the original workbook, which meant that there was a difference in the number of records and the number of unique genes. Three experimental conditions were collected as root samples while two experimental conditions were collected as leaf samples (Figure 1). These characteristics laid the groundwork for a description of ARF-related expression patterns in different tissues and experimental groups and are summarized in Table 1.

Table 1. Transcriptomic Dataset Characteristics and ARF Gene Distribution

Parameter	Root	Leaf	Total
Samples	9	6	15
Experimental groups	3	2	5
Samples per group	3	3	3
Gene records	124	124	124
Unique gene IDs	90	90	90

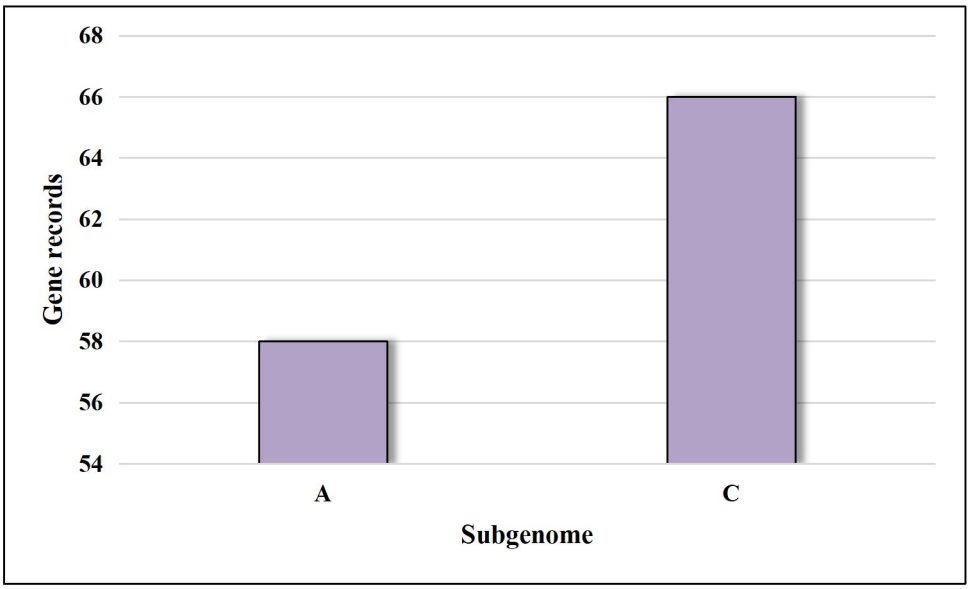

Figure 1. Chromosomal Subgenome Distribution of ARF-Related Gene Records

3.2. Preprocessing Outcomes and ARF Expression Characteristics

In the pre-processing, extensive differences in expression measurements for ARF were found between the experimental groups (Figure 2). The mean value for the expression was 7.999 in the control root group and 4.573 in control leaf samples. The Z12 root group had the greatest overall mean expression (13.144) while

the Z4 root group had the lowest mean expression of 3.860. The mean expression for the Leaf Z samples was 4.438. These differences in expression descriptions suggested that there were different distributions across tissues and treatments, however there was still a need for expression scale verification of the direct biological interpretation (Table 2).

Table 2. Descriptive Statistics of ARF Gene Expression Across Experimental Groups

Group	Samples	Mean	Median	SD
Root CK	3	7.999	5.470	8.488
Root Z12	3	13.144	4.850	20.451
Root Z4	3	3.860	2.558	5.023
Leaf CK	3	4.573	2.490	5.927
Leaf Z	3	4.438	1.690	7.377

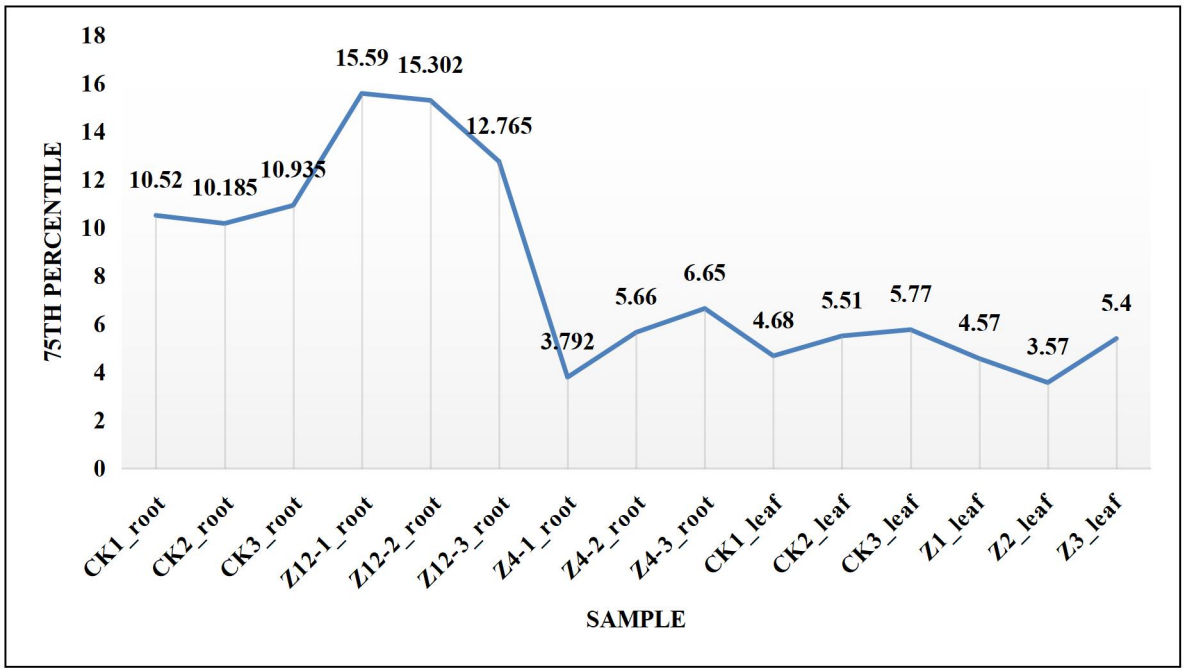

Figure 2. Sample-Level Expression Distribution of ARF Genes

3.3. Differential Expression and Tissue-Enriched ARF Genes

There was significant difference in expression of some ARF related genes between control root and leaf tissues. Some genes showed increased mean values in roots, such as BnaA05G0014400ZS and BnaC04G0014300ZS. Other genes showed relatively minimal tissue associated differences (Figure 3). The spatial distribution of transcriptional activity implicated by the observed patterns indicated that there may be differential expression of ARF-related genes between the tissues sampled. These differences, however, were expression contrasts only, and not established as differential expression by a statistical test. The selected candidate genes and their expression in the control groups are listed in Table 3 for further study.

Table 3. Comparative Expression of Selected ARF-Related Genes

Gene ID	Root CK	Leaf CK	Root/Leaf ratio
BnaA05G0014400ZS	8.46	0.16	52.88
BnaC04G0014300ZS	8.46	0.45	18.80
BnaA07G0370400ZS	1.56	0.20	7.80
BnaC07G0199900ZS	12.85	1.42	9.05
BnaA07G0137300ZS	12.23	1.14	10.73

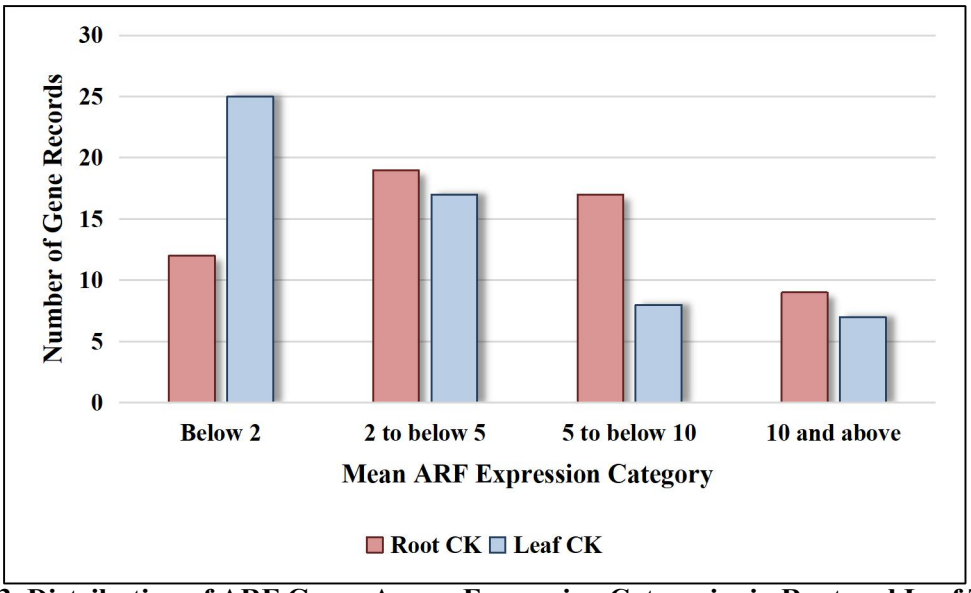

Figure 3. Distribution of ARF Genes Across Expression Categories in Root and Leaf Tissues

3.4. Hierarchical Clustering and Principal Component Analysis

The expression matrix showed that there were differences in the completeness of data between experimental groups, thus affecting the data for use in Hierarchical Clustering and Principal Component Analysis. Complete numerical observations were found for all the root and Z12 root samples, but their corresponding measurements were missing for the Z4 root and Z4 leaf. This could impact distance calculations, stability of clusters, and multivariate sample positioning. As a result, the expression structure of the tissue could not be reliably determined based on unprocessed measurements. Table 4 presents an overview of numerical observations available and percentage of missing data for subsequent multivariate analysis.

Table 4. Expression Matrix Completeness for Multivariate Analysis

Group	Expected values	Available	Missing	Missing (%)
Root CK	372	372	0	0.00
Root Z12	372	372	0	0.00
Root Z4	372	238	134	36.02
Leaf CK	372	171	201	54.03
Leaf Z	372	171	201	54.03
Total	1,860	1,324	536	28.82

3.5. Functional Annotation and Biological Pathway Associations

The 124 ARF related gene records were found to have different degrees of coverage for functional annotation. 87 records were identified with KO identifiers, 57 records were identified with gene description and Swiss-Prot annotations (Figure 4). Of the 50 records, 50 had information on Gene Ontology, and 20 had information on KEGG pathway annotations. These results indicated that only a small portion of the data set was functionally characterized. The available annotations enabled the study of some auxin-related regulatory functions, while others were not enriched and needed to be checked (Table 5).

Table 5. Functional Annotation Coverage of ARF-Related Gene Records

Annotation	Available	Unavailable	Coverage (%)
Gene description	57	67	45.97
GO	50	74	40.32
KO ID	87	37	70.16
KO gene	87	37	70.16
KEGG pathway	20	104	16.13
Swiss-Prot ID	57	67	45.97
Swiss-Prot annotation	57	67	45.97

KOG ID	0	124	0.00

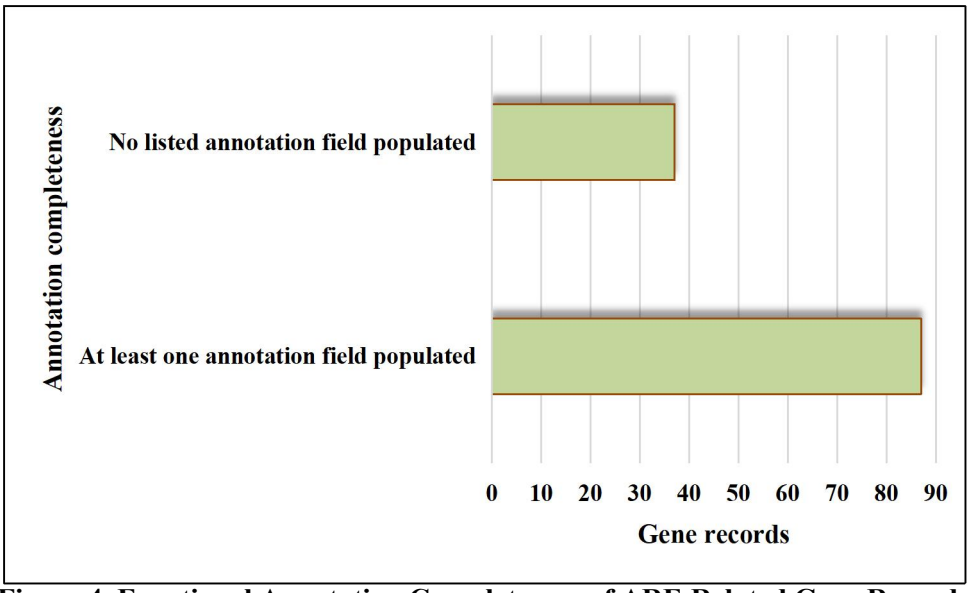

Figure 4. Functional Annotation Completeness of ARF-Related Gene Records

4. Discussion

Comparative transcriptomic analysis of root and leaf tissue revealed tissue associated expression patterns of genes related to Auxin Response Factor (ARF) in the context of *Brassica napus*. The analysis resulted in 124 gene records with 90 distinct gene identifiers, underscoring the diversity of transcriptional responses in the dataset related to ARF. The differences in expression in various tissues and conditions indicated that there could be tissue-specific variation in auxin-related regulatory pathways. The results are in line with previous transcriptomic studies, which showed that the regulation of gene expression is highly differential between plant tissues and developmental situations. The waterlogging stress caused distinct molecular responses that related to tissue function and environmental adaptation, highlighting the importance of tissue level transcriptomic analysis, as identified in B. napus (Hong et al., 2023). Likewise, genome-wide studies of transcription factor families have revealed distinct tissue-specific expression patterns, suggesting that different regulatory genes play varying roles in developmental and physiological events in rapeseed (Pu et al., 2025).

There were differences in expression between the root and leaf controls, with mean expression values of 7.999 and 4.573, respectively. The expression of several candidate genes, such as BnaA05G0014400ZS and BnaC04G0014300ZS, was found to be higher in the roots than in the leaves. The results indicate some possible correlations between ARF activity and physiological needs of the roots. Hormonal regulation, nutrient uptake, cell fate determination and environmental sensing are essential for root development to occur in an orchestrated manner. In previous transcriptomic studies of potassium utilization efficiency in B. napus, regulatory genes that control root growth and nutrient responsive developmental processes were identified which is indicative of the role of transcriptional control in the root physiological adaptation to K deficiency (Ibrahim et al., 2023). In addition, plant structure studies have revealed a list of candidate genes involved in developmental control, indicating that the difference in transcription levels could be responsible for the variation in plant architecture of rapeseed (Ye et al., 2022). However, the genes that are expressed differently in the present study should still be subjected to more statistical and experimental investigations before it is determined whether they can be considered as genes that are specifically expressed in the root.

The differences in the responses between the experimental groups also indicated different transcriptional responses. The Z12 root group had the highest mean expression value of 13.144, while the Z4 had a lower mean of 3.860. Such differences may be caused by condition dependent regulation, but the biological significance of the treatment labels should be clarified. Auxin signaling is especially important because alterations in auxin metabolism may effect downstream transcriptional events and possible developmental events. In *Brassica rapa*, long-chain auxin conjugates were used to investigate how auxin metabolism affects the genome. Gene expression dynamics are associated with changes in auxin metabolism, suggesting a link between the regulation of auxin and changes in the genome (Smolko et al., 2023). Similarly, the expression of members of transcription factor families varied under abiotic stress in genome-wide analyses of TCP transcription factors in rapeseed (Xu et al., 2025). Such results indicate the potential for expression

differences within experimental groups to be due to regulatory differences and not due to consistent responses.

Considering the distribution of expression categories further revealed differences between root and leaf tissue. The number of records in intermediate expression ranges was higher in root control samples than in leaf control samples, with higher numbers represented in the lowest expression range in leaf control samples. Expressed in these observations are differential transcriptional activity that may reflect tissue-specific developmental functions. The distributions should be interpreted with caution, however, because of differences in the extent of missing data coverage. In addition, tissue-specific molecular responses have been observed for P deficiency, which involved tissue-specific hormonal regulation with root, and metabolic remodeling with leaf tissue, resulting in different adaptation strategies in *Brassica rapa* (Zhang, 2025). For example, transcriptomic network analysis of broccoli under salt stress revealed tissue-specific and temporal regulation responses, highlighting the necessity of tissue identity and environmental context in understanding gene regulation under salt stress (Yepes-Molina et al., 2025). All these studies offer a comparative context for the interpretation of the variation in expression of ARF, and none assume that all such variation is a direct consequence of auxin signaling.

In the replicate expression profile analysis, the Pearson correlation coefficients between experimental groups were generally good, ranging from 0.783 to 0.990. These correlations showed considerable agreement between the replicate measurements but some differences between the individual samples. This consistency facilitates the reproducibility of the expression pattern description without excluding the possible contaminating effect of the technical and/or biological factors. To elucidate the potential roles of these genes, combining identification and expression analysis of these genes allowed for a more comprehensive understanding of the basic pentacysteine transcription factor's role in B. napus (Hu et al., 2025). Similarly, the expression variation of the BoBZR1 genes within the gene family was depicted in *Brassica oleracea* for regulatory gene characterization, which further underlines the significance of comparative transcriptomic analysis (Ullah et al., 2025).

The results from functional annotation assessment showed that the information of functional annotations is not complete: 50 records from Gene Ontology and 20 records from the KEGG pathway. This resulted in some biological background information, although not extensive, via these annotations, but due to the sparse availability of these annotations, comprehensive pathway-level interpretation was limited. Nitrogen stress-induced plant regulation has been studied by integrated multi-omics analysis in *Brassica rapa*, and it has been found that combining transcriptomic data with other complementary molecular measurements can enhance the understanding of the regulation of nitrogen stress (Gong et al., 2025). In addition, physiological and genetic studies of heat stress in B. napus have focused on the complexity of plant responses and the need to incorporate molecular data with physiological evidence (Kourani et al., 2022). Therefore, conclusions based on the results of the present study must be interpreted as preliminary results of variation in expression of ARF in tissues. Further studies with validated expression measurements, balanced experimental comparisons, extended functional annotations and independent molecular validation may elucidate the role of the identified candidate genes in regulation and enhance understanding of the auxin associated transcriptional organization in *B. napus.*

5. Conclusion

Comparative transcriptomic profiling and functional annotation of expression patterns of genes related to Auxin Response Factors (ARFs) in root and leaf tissue of *Brassica napus*. The analysis revealed 90 distinct gene identifiers and 124 gene records showing significant differences in expression in different tissues and under different experimental conditions. The average expression value of root control samples was higher than the average value of leaf control samples, and root-enriched expression was observed for several candidate genes, such as BnaA05G0014400ZS and BnaC04G0014300ZS. Further differences between experimental groups also supported the idea of the different transcriptional responses, indicating that tissue-specific regulatory processes might involve ARF-related genes. Generally consistent expression patterns were observed by replicating the correlation analysis, which is descriptive and reliable. The functional annotation gave initial clues to possible biological functions, but due to incomplete annotation coverage, extensive pathway interpretation was not possible. The results indicate the significance of tissue-specific transcriptional regulation in the comprehension of auxin related developmental processes in B. napus. However, conclusions about differential expression and regulatory mechanisms are subject to certain uncertainties owing to normalization in expression, missing measurements, and experimental conditions. The use of validated transcriptomic analysis, independent biological samples, and functional characterization in further investigations could explain the roles of the candidate ARF genes in root and leaf

development. Overall, the results of this study suggest a preliminary molecular basis for investigating tissue-associated auxin regulation and for future research in functional genomics and crop improvement.

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