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Gene Expression Analysis of *IND*, *ALC*, *RPL*, and *FUL* in Reducing Seed Shattering in Flixweed (*Descurainia sophia*)

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ABSTRACT

The indeterminate growth habit and asynchronous pod maturation in flixweed (*Descurainia sophia*) lead to seed shattering and a consequent reduction in economic yield. Genes involved in the formation and differentiation of the dehiscence zone play a pivotal role by promoting or suppressing valve margin tissue identity. In this study, the expression of four key genes— *INDEHISCENT (IND)*, *ALCATRAZ (ALC)*, *REPLUMLESS (RPL)*, and *FRUITFULL* (*FUL*) —was investigated under two plant hormone treatments (indole-3-butyric acid (IBA) and gibberellic acid (GA)), each applied at four different concentrations. Among these genes, *IND* and *ALC* promote seed shattering, whereas *RPL* and *FUL* function as negative regulators of pod dehiscence. In addition, six quantitative traits and their correlations with the expression of target genes were evaluated. The results showed that both IBA and GA influenced seed shattering by directly modulating the expression of genes responsible for the differentiation of the lignification layer (LL) and separation layer (SL). IBA at 40 mg L^{-1} was identified as the most effective treatment for reducing seed shattering in flixweed. Correlation analyses revealed significant relationships ($r=0.31$-0.58, $p<0.05$) between the expression of *FUL* and *RPL* with seed yield and harvest index, whereas *IND* showed mainly negative correlations with thousand-seed weight ($r=-0.34$) and plant height ($r=-0.15$). Co-expression network analysis highlighted *HEC3*, a transcription factor involved in valve margin development, and *RPL* as central regulators in the genetic control of seed shattering. Plants treated with IBA 40 mg L^{-1} exhibited the highest harvest index, increased expression of the seed-retention genes *FUL* and *RPL*, and reduced expression of the shattering-promoting genes *IND* and *ALC*. Although GA at 15 mg L^{-1} represented the most effective treatment, IBA at 40 mg L^{-1} consistently produced superior improvements in growth, yield, and seed shattering reduction.

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1. Introduction

Flixweed (*Descurainia sophia*), also known by its synonyms *Sisymbrium sophia* and *Sisymbrium irio*, is an annual or biennial herb belonging to the family *Brassicaceae*. The plant can reach up to one meter in height, and its small, slightly elongated seeds are enclosed within slender pods that occur in two distinct colors (Lehnhoff *et al.*, 2025). A major agronomic challenge in flixweed cultivation is the asynchronous maturation of pods along the main stem, which leads to seed shattering and consequently lowers economic yield. In many plant species, the inflorescence meristem remains active after the transition from the vegetative to the reproductive phase, continuously producing flowers and fruits. This growth pattern, known as indeterminate flowering/inflorescence, is characterized by a prolonged activity of the apical meristem, which keeps generating floral organs even after fruit set. In contrast, determinate inflorescence terminates after a fixed number of flowers, as the apical meristem differentiates into a terminal flower. Flixweed follows an indeterminate flowering pattern, typically blooming from late May to early July (Balanzà *et al.*, 2019; Lehnhoff *et al.*, 2025). Genes involved in the formation and regulation of the pod dehiscence zone are critical for pod development and

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seed retention. Among these, *FUL*, *SHP1*, *SHP2*, *ALC*, *IND*, and *RPL* are of particular importance. The genes *SHATTERPROOF1 (SHP1)*, *SHATTERPROOF2 (SHP2)*, *IND*, and *ALC* enhance pod opening by promoting differentiation of the dehiscence zone, while *FUL* and *RPL* act antagonistically by suppressing shattering through their effects on valve margin cells. Thus, changes in the expression of these genes directly influence the degree of seed shattering in flixweed. Reducing seed shattering must be achieved without compromising seed size or number, as pod retention alone does not guarantee maximum yield.

Pod dehiscence is regulated by a well-characterized genetic network in which several key transcription factors act in a coordinated manner. *FUL* functions as an upstream regulator that suppresses the expression of *SHP1* and *SHP2* in the valve tissues, thereby restricting dehiscence zone identity to the valve margin (Bemer *et al.*, 2017; Hou *et al.*, 2013). Whereas *SHP* genes do not exert reciprocal regulation on *FUL*, establishing a unidirectional regulatory hierarchy. This hierarchy provides a suitable framework to investigate whether exogenous application of IBA and GA can modulate pod dehiscence by altering the expression balance between upstream *FUL* and downstream *SHP-IND/ALC* components of the network. In full mutants, the natural dehiscence zone fails to differentiate. Lignification pattern analysis of the fruit margin revealed that, whereas wild-type siliques possess a single lignified valve cell layer, ful mutants show lignification throughout the entire valve tissue (Ferrándiz and Fourquin, 2014; Liu *et al.*, 2016). In Arabidopsis, *SHP1* and *SHP2* promote the differentiation of valve margin cells by activating downstream regulators such as *IND* and *ALC*, which are essential for proper formation of the separation and lignification layers (Ehlers *et al.*, 2016). Single mutations in *shp1* or *shp2* have little phenotypic effect, but double mutants produce non-dehiscent pods, indicating additive gene function. Unlike wild-type fruits, where lignification is observed in valve margin cells adjacent to the dehiscence zone, *shp1*/*shp2* mutants show reduced lignification. *FUL* negatively regulates *SHP1* and *SHP2* expression, whereas *SHP* genes have no reciprocal effect on *FUL*. These observations suggest that *SHP* genes are activated by *AGAMOUS* (*AG*) while *FUL* acts as a repressor of *SHP* expression (Deng *et al.*, 2011; Dreni and Kater, 2014; Hanano and Goto, 2011). *RPL* acts antagonistically to SHP genes by maintaining replum identity and spatially confining the dehiscence zone (Sun *et al.*, 2023). Unlike *shp1*/*shp2* mutants that disrupt differentiation of the entire dehiscence zone, alc mutants exhibit normal external morphology and lignification patterns, affecting only a subset of valve margin tissues (Rajani and Sundaresan, 2001). Together, the FUL–SHP–IND/ALC regulatory module establishes precise spatial control over valve margin development, ultimately determining pod shattering behavior (Fig. 1).

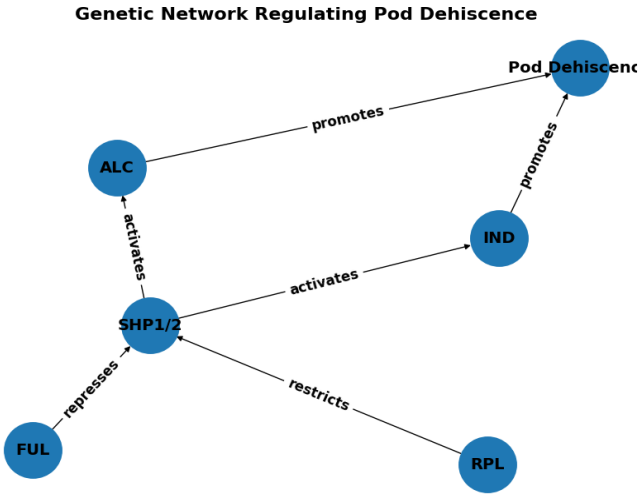


Figure 1. Schematic representation of the genetic regulatory network controlling pod dehiscence in Sisymbrium spp. The transcription factors *SHP1/2* act as central regulators by activating *IND* and *ALC*, which in turn promote pod dehiscence through proper differentiation of the valve margin tissues. *FUL* negatively regulates *SHP1/2*, thereby restricting premature dehiscence, while *RPL* contributes to replum identity and limits the spatial expansion of the dehiscence zone. Arrows indicate activation or promotion, and blunt-ended lines indicate repression or restriction.

Given the importance of these genetic pathways, changes in the expression of pod-dehiscence genes strongly influence seed shattering (Zhang *et al.*, 2020). However, emerging evidence suggests species-specific variations in gene function, indicating that functional validation in flixweed is still required (Lotz *et al.*, 2024). Plant hormones such as auxins are known to influence the expression of these bHLH genes and their downstream targets (Song *et al.*, 2022). The *INDEHISCENT* (*IND*) gene encodes a basic helix–loop–helix (bHLH) transcription factor that specifies valve margin identity and plays a critical role in the differentiation of the lignification layer (LL) and separation layer (SL) (Liljegren *et al.*, 2004). Conversely, *REPLUMLESS* (*RPL*) encodes a

homeodomain protein that functions similarly to *FUL* by negatively regulating valve margin identity genes within the replum (Xu *et al.*, 2022). Auxins, through their roles in plant growth and tissue lignification, may affect the expression of these key regulators. Indole-3-butyric acid (IBA) was selected as the auxin treatment due to its greater chemical stability and more consistent physiological effects under exogenous application compared with IAA (Yang *et al.*, 2025). Together, the combined evaluation of gibberellin and IBA provides an appropriate hormonal framework to investigate the regulation of seed shattering and yield-related traits in flixweed. This study aimed to identify optimal concentrations of IBA and GA to enhance flixweed yield through physiological regulation and modulation of key pod-dehiscence genes.

2. Materials and methods

This experiment was conducted in the research fields of the Research Institute of Zabol (UTM-x=377140.224, y=3412299.207) using a completely randomized design with three replications. Two plant growth regulators were applied: IBA at concentrations of 10, 20, 30, and 40 mg L^{-1}, and GA at concentrations of 15, 30, 45, and 60 mg L^{-1}. These concentration ranges were chosen to encompass suboptimal to optimal levels reported in the literature, allowing the identification of dose-dependent responses in growth, yield, and seed shattering (Kalkali *et al.*, 2023; Montazerinezhad *et al.*, 2024; Paam *et al.*, 2024; Ranjbar *et al.*, 2024; Shiri *et al.*, 2020). IBA and GA were applied in separate experimental treatments, allowing evaluation of their individual effects. Foliar sprays were performed at two growth stages—seedling and flowering—using the specified concentrations. Control plants were sprayed with distilled water. At physiological maturity, 20 plants were randomly selected from each plot to measure quantitative traits.

To examine the expression of the four key genes (*IND*, *ALC*, *RPL*, and *FUL*)—among which *IND* and *ALC* promote seed shattering, while *RPL* and *FUL* suppress it—four pairs of gene-specific primers were designed based on mRNA sequences retrieved from the NCBI database, using Primer3 Plus and CLC Main Workbench v7 (Table 1). *Actin7* was used as the reference gene.

Table 1. Sequences of primers used in the PCR reaction. The ACT7 gene was used as a reference gene.

Gene name	Gene ID		Primers 5'-3'	Product length (bp)
FUL	XM_024151164.1	Fw	TGGTCATTTCAGGGTTGTCA	155
		Rv	ACCAGACCTTCGCTTTGAGA	
RPL	XM_006398603.2	Fw	CGGACCAGCTTCAAGTTAGC	134
		Rv	TTGACCAGCAGAACACAAGC	
IND	XM_006396147.2	Fw	AGTTCTTGAAACGGCAGGTG	188
		Rv	CAATCGTCGATCGAAATCCT	
ALC	XM_006393847.2	Fw	TGAGGCCTTACAGCCAGAGT	157
		Rv	GCCGAATCATCACATCACTG	
Actin7	XM_006399401.2	Fw	TTCCCAAGTATTGTTGGTCGTC	168
		Rv	CCAGATCTTCTCCATGTCATCC	

Total RNA was extracted following the method of Japelaghi (Japelaghi *et al.*, 2011). RNA quality and quantity were assessed by spectrophotometry and horizontal gel electrophoresis, and suitable samples were used for cDNA synthesis. Using a thermal cycler with gradient temperature settings, the optimal annealing temperature for each gene was determined. Quantitative real-time PCR was subsequently performed (in three biological and three technical replicates) with a Rotor-Gene 3000 system using a 2× Taq Master Mix PCR kit (CinaGene, Iran). Relative expression levels were calculated using the ΔΔCT method (Livak and Schmittgen, 2001). To construct the gene co-expression network, interaction data were retrieved from the STRING v11 database (Szklarczyk *et al.*, 2023). Co-expression relationships were inferred using the co-expression evidence channel implemented in STRING v11. Network visualization was carried out using Cytoscape v3.7.1 software (Ono *et al.*, 2025).

Quantitative traits, including harvest index, number of dehisced pods, seed yield, thousand-seed weight, plant height, and number of seeds per pod, were analyzed. The experiment was conducted as a completely randomized design (CRD) with three biological replicates. Data were subjected to analysis of variance (ANOVA) using SAS software (v. 9), and

mean comparisons were performed using an appropriate post-hoc test at $p<0.05$. Correlations between gene expression levels and quantitative traits were evaluated using Pearson's correlation coefficient.

An additional parameter measured was the proportion of dehisced pods per plant. Because the total pod number varied among plants, the proportional measure of dehisced pods was calculated using the ratio of dehisced pods to total pods (Equation 1).

$$x = \frac{ab}{c} \quad (1)$$

Where x represents the ratio of dehisced pods to the total number of pods, a is the total number of examined plants (20 plants per treatment), b is the total number of split pods, and c is the total number of examined pods.

3. Results and discussion

While new pods continue to form along the upper parts of the flowering stem, the lower pods mature and eventually dehisce, releasing their seeds (Fig. 2). Such asynchronous maturation leads to substantial seed loss—up to 30% of potential yield (Takahashi *et al.*, 2020)—causing not only reduced economic return but also the emergence of volunteer seedlings in subsequent seasons and complicating harvest timing.

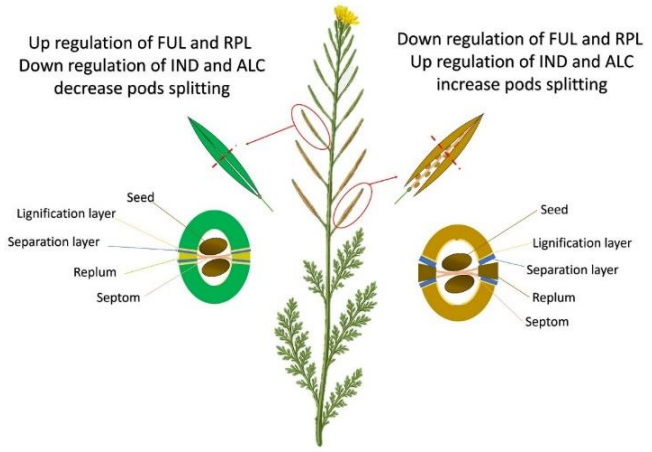


Figure 2. Over a period of 3 to 4 weeks, Flixweed continuously produces new pods while the older pods split open and release their seeds. A cross-section of two dehisced pods and one intact pod clearly shows the differentiated cellular layers in the pod wall. Four key genes, by regulating the differentiation of these cells, control the process of pod dehiscence.

Plant hormones can modify pod dehiscence by influencing the expression of genes associated with pod opening. They also affect other quantitative traits such as plant height, seed number per pod, and thousand-seed weight. Therefore, reducing seed shattering must be achieved without compromising seed size or number, as pod retention alone does not guarantee maximum yield.

3.1. Quantitative trait analysis

Six quantitative traits—plant height, seed number per pod, thousand-seed weight, seed yield, and harvest index—were analyzed to identify the most effective hormone and concentration (Fig. 3). IBA treatments (10–40 mg L^{-1}) significantly increased plant height compared with the control, with the greatest effect observed at 40 mg L^{-1} (Fig. 3a). By contrast, GA treatments, particularly at 60 mg L^{-1}, caused a marked reduction in plant height. These findings are consistent with previous reports describing auxins as strong stimulators of shoot elongation and vegetative growth (Lestari *et al.*, 2024).

Gibberellins are generally recognized as key regulators of internode elongation (Dai *et al.*, 2025); however, plant responses to GA are non-linear, and high concentrations can trigger negative feedback mechanisms such as induction of GA-catabolic genes (e.g., GA2ox) or hormonal imbalance with auxins, resulting in growth inhibition (Hedden and Sponsel, 2015). The inhibitory effect of high GA concentrations was also evident in seed number per pod (Fig. 3b). Similar non-linear responses have been reported in other species: for example, in okra, GA application increased plant height but had little effect on flower initiation or pod formation (Mohammadi *et al.*, 2014); in soybean, pod set is regulated by GA biosynthesis during post-flowering vegetative growth (Taniguchi *et al.*, 2018); and in cauliflower (*Brassica oleracea*), GA at 200 ppm slightly increased seed number, while higher concentrations significantly reduced it (Prodhan *et al.*, 2022).

Collectively, these studies confirm the dose-dependent and potentially inhibitory effects of GA (Abdel-Mola *et al.*, 2022; Kalkali *et al.*, 2023; Montazerinezhad *et al.*, 2024; Paam *et al.*, 2024; Ranjbar *et al.*, 2024; Shiri *et al.*, 2018, Shiri *et al.*, 2020). It should be noted that plant responses to gibberellic acid are highly species-specific and may vary depending on developmental and physiological context.

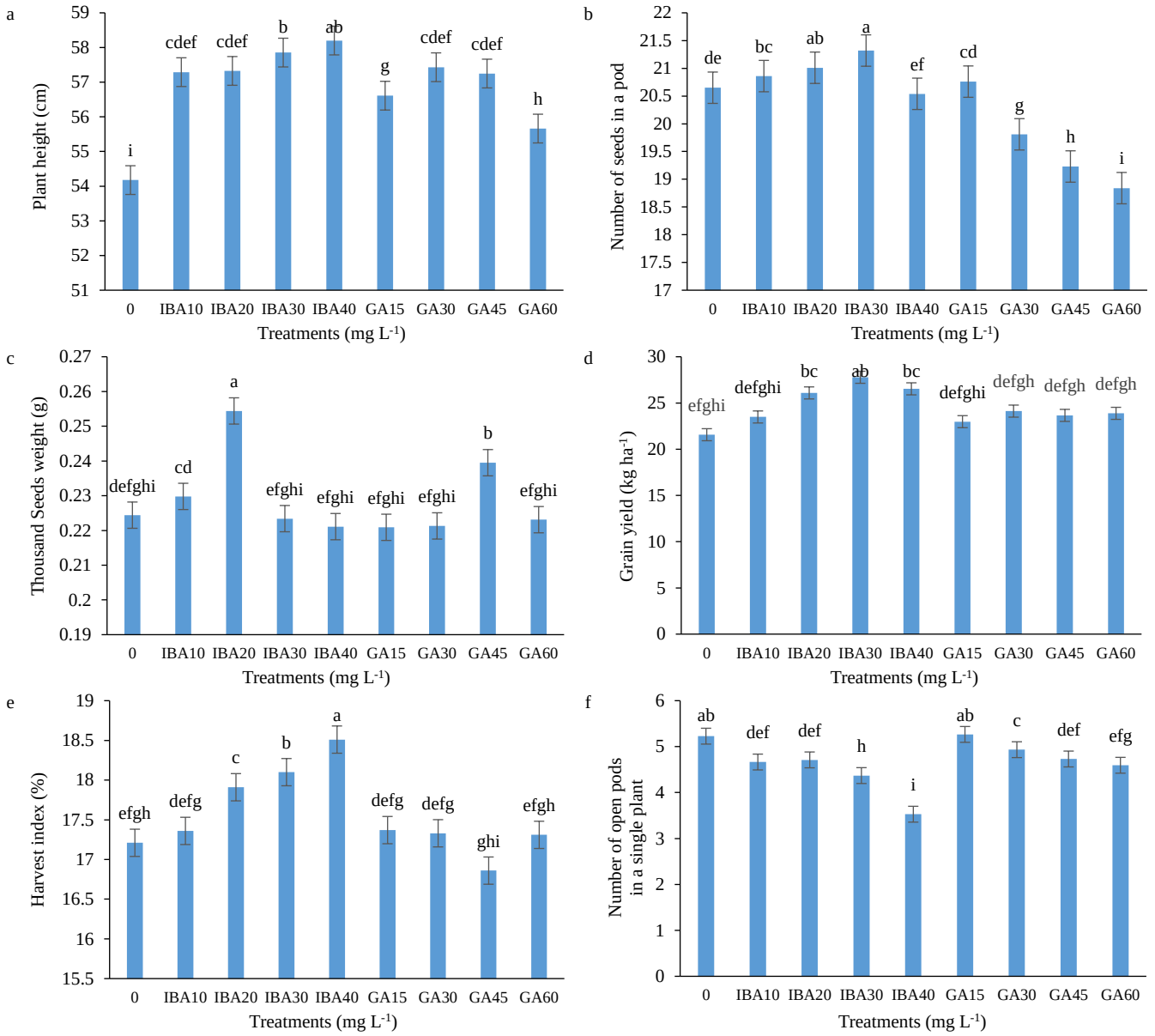


Figure 3. Graphs of the quantitative traits examined under IBA and GA treatments in flixweed. The traits are: a. plant height, b. number of seeds per pod (Averrage number counted), c. thousand-seed weight, d. grain yield, e. harvest index, and f. number of open pods per plant (Average number counted). The IBA treatment had a greater enhancing effect on growth and yield-related indices compared to GA.

Although most previous studies have focused on the role of IBA in rooting and plant establishment, some evidence supports its positive influence on seed yield. For example, IBA spraying improved seed production in onion by increasing seed number per fruit (Gabri *et al.*, 2024), which agrees with the present results. Overall, the growth and yield indices clearly demonstrate that IBA treatments outperformed GA treatments (Fig. 3). Similar positive effects of IBA on vegetative growth and yield have been reported in rapeseed, where IBA enhanced stem length, assimilate accumulation, and final seed yield (Li *et al.*, 2024; Li *et al.*, 2018). In contrast, GA responses are less predictable and depend strongly on concentration, timing, and application method; in some cases, GA sprays negatively affect seed production by disturbing floral organ development (Castro-Camba *et al.*, 2022).

Hormone treatments significantly reduced pod dehiscence compared with the control, with the greatest reduction observed in plants treated with IBA 40 mg L^{-1}. This finding indicates that optimal IBA concentration effectively prevents pod splitting—a key factor in minimizing yield loss. Reducing seed shattering must be achieved without compromising seed size or number, as pod retention alone does not guarantee maximum yield. Auxins and their derivatives are known to increase adhesion of valve margin cells and interfere with tissue differentiation and lignification

processes, thereby enhancing pod integrity and seed retention (Dong and Wang, 2015; Liu *et al.*, 2024). Auxin treatments likely reduce pod dehiscence by reinforcing valve margin cell adhesion through coordinated transcriptional regulation. The upregulation of *FUL* and *RPL* suppresses the expression of the bHLH genes *IND* and *ALC*, which are required for differentiation and separation of the valve margin. This regulatory shift limits the formation of a functional separation layer, thereby reducing cell wall loosening and preventing premature pod opening.

3.2. Expression analysis of target genes

The fruits of Arabidopsis, rapeseed, and other Brassicaceae members are siliques composed of two valves attached to a central replum through a specialized valve margin tissue. The valve margin contains two distinct layers: a lignification layer (LL), which provides mechanical tension, and a separation layer (SL), which secretes polygalacturonases to degrade cell walls and allow cell separation (Arnaud *et al.*, 2010). Genes *IND* and *ALC* promote differentiation of LL and SL, respectively, and their increased expression facilitates pod opening. Conversely, *FUL* and *RPL* suppress the action of *IND* and *ALC*, thereby reducing pod dehiscence (Singh *et al.*, 2024).

Previous studies have shown that auxin and gibberellin independently influence the expression of genes involved in LL and SL differentiation. In Arabidopsis, *IND* acts as a central coordinator of multiple hormonal pathways (Simonini *et al.*, 2017). In the present study, we observed that *IND* expression responded to exogenous IBA and GA treatments, although not in a strictly dose-dependent manner. This responsiveness suggests that *IND* may act as a downstream convergence point for hormone-mediated regulatory signals in flixweed. While our data do not directly dissect the molecular crosstalk mechanisms, the observed transcriptional modulation of *IND* under hormone treatments supports its potential role as an integrative node within the regulatory network. GA biosynthesis, enhanced by *IND*, is required for silique opening and seed dispersal (van Gelderen *et al.*, 2016). Localized auxin production is also essential for SL differentiation (Li *et al.*, 2019). Exogenous hormone treatments can therefore supply sufficient hormone levels to downregulate the expression of genes required for endogenous hormone biosynthesis, but plant responses remain highly non-linear (Maity *et al.*, 2021).

As shown in Fig. 4, IBA treatments increased the expression of *FUL* and *RPL* in a concentration-dependent manner, consistent with the observed reduction in pod dehiscence. In contrast to *FUL* and *RPL*, whose expression showed a clear dose-dependent increase under rising IBA concentrations and was consistently associated with a reduction in pod shattering, the expression patterns of *IND* and *ALC* did not follow this monotonic trend. Instead, their transcriptional responses varied across hormone concentrations and were not directly aligned with the observed decrease in the number of dehisced pods. Moya-Cuevas et al. (2024), through transcriptomic modeling analysis, confirmed the repressive roles of *FUL* and *RPL* on the *SHP*/*IND*/*ALC* module and the promotive roles of *IND* and *ALC* in SL formation, supporting the present findings (Moya-Cuevas *et al.*, 2024).

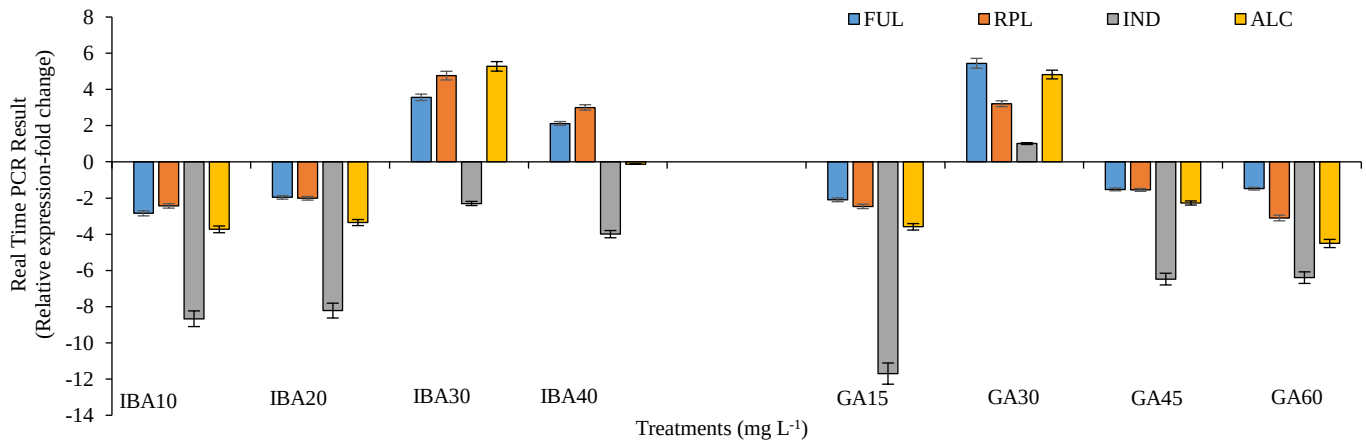


Figure 4. Comparison of the mean relative expression of genes involved in the pod development of Flixweed under the PGRs treatments of GA and IBA. By upregulation of *FUL*/*RPL* and downregulation of *IND*/*ALC*, IBA at 40 mg L^{-1} emerged as the most effective treatment for reducing seed shattering in flixweed.

3.3. Correlations between gene expression and quantitative traits

Correlation analysis revealed that *FUL* and *RPL* showed the strongest positive associations with seed yield and harvest index, while *IND* exhibited negative correlations with thousand-seed weight and plant height (Fig. 5). These relationships underscore the potential of *FUL* and *RPL* as enhancers of yield and harvesting efficiency, and the limiting role of *IND*. Similar patterns have been reported in Arabidopsis and other Brassicaceae species, where *FUL* promotes fruit tissue differentiation and seed filling (Di Marzo *et al.*, 2020), and *RPL* enhances silique growth and seed production capacity (Roeder *et al.*, 2003). Conversely, *IND* is a key activator of pod opening and seed dispersal, and *ALC* participates in seed release and fruit maturation (Ivarson *et al.*, 2023). These results suggest that suppression of *IND* expression could be a promising breeding strategy to reduce seed shattering and improve yield stability in flixweed.

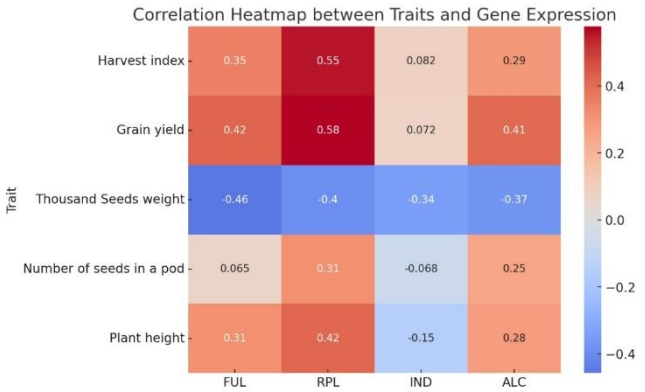


Figure 5. Heatmap diagram showing the correlation of the expression of four genes, *FUL*, *RPL*, *IND* and *ALC* with changes in the quantitative traits in the flixweed. The color intensity from blue (negative correlation) to red (positive correlation) indicates the direction and magnitude of the correlation coefficient. As can be seen, the expression of *RPL* and *FUL* has the highest positive correlation with harvest index and grain yield, while thousand-grain weight shows a significant negative correlation with most genes.

Interestingly, thousand-seed weight showed a negative correlation with *IND* and *ALC* expression, which are known promoters of pod shattering. Although this relationship appears counterintuitive, it may reflect a developmental trade-off. Elevated expression of *IND* and *ALC* could accelerate valve margin differentiation and separation layer formation, potentially reallocating assimilates toward structural remodeling processes rather than seed filling. Alternatively, higher activity of these shattering-related genes may be associated with earlier pod maturation, thereby shortening the effective seed-filling period. Further functional analyses are required to clarify this relationship.

3.4. Gene co-expression network analysis

Network reconstruction revealed a central role for *HEC3* in flixweed pod maturation (Fig. 6). Topological analysis using betweenness centrality (BC) and closeness centrality (CC) identified *HEC3* and *RPL* as hub genes with the highest BC value (0.0714) (Table 2).

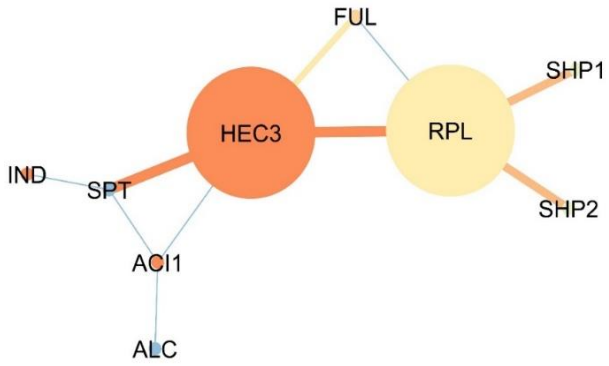


Figure 6. Gene network of the studied genes involved in seed shattering in flixweed. The size of each node represents the CC value (larger nodes indicate higher values), and the color of each node represents the BC value (nodes with higher values are closer to red, while lower values are closer to blue). Similarly, thicker and more reddish edges indicate higher Edge Betweenness (EB) values. The network topology suggests that the HEC3 gene plays an important role in the maturation process of flixweed siliques.

Table 2. Topological analysis of coexpression network of genes involved in Flixweed shattering seed

Name	Edge count	Betweenness centrality	Closeness centrality
HEC3	4	0.071429	1
RPL	4	0.071429	0.666667
ACI1	3	0	1
SPT	3	0	0
FUL	2	0	0.75
ALC	1	0	0
SHP1	1	0	0.5
SHP2	1	0	0.5
IND	1	0	1

The prominent network position of *HEC3* is noteworthy. Although *ACI1* and *IND* also showed high CC values, *HEC3* and *RPL* were distinguished by their simultaneous prominence across all evaluated parameters. Therefore, our interpretation emphasizes these two genes as principal hubs within the network. Members of the *HECATE* (*HEC1/2/3*) family are bHLH transcription factors expressed in the female reproductive tissues of the gynoecium (Arias-Pérez *et al.*, 2025). These regulatory roles have been associated

with the establishment of transmission tissues and hormone gradients required for activation of *IND* and *ALC* at the valve margin (Schuster *et al.*, 2015). In the present study, this connection is interpreted in light of previous findings, as hormone gradients were not directly quantified. *HEC3* was not included as an initial candidate gene and, to our knowledge, has not been directly implicated in pod shattering in previous studies. However, it emerged as a central hub in the unbiased co-expression network constructed from our dataset. Given its prominent network connectivity, *HEC3* may represent a previously unrecognized regulatory component associated with pod dehiscence in flixweed. This observation should be considered hypothesis-generating and requires further functional validation. Although the genetic network controlling pod dehiscence has been extensively characterized in Arabidopsis, its functional relevance in flixweed remains largely unexplored. Given the distinct growth habit and agronomic importance of flixweed, functional validation of this network in this species is required.

4. Conclusion

Hormonal regulation significantly influences pod dehiscence and, consequently, the final yield of flixweed (Descurainia sophia). In this study, two plant growth regulators—indole-3-butyric acid (IBA) at concentrations of 10, 20, 30, and 40 mg L^{-1} and gibberellic acid (GA) at concentrations of 15, 30, 45, and 60 mg L^{-1}—were applied to evaluate their effects on pod shattering and associated gene expression. Five key agronomic traits (plant height, seed number per pod, thousand-seed weight, seed yield, and harvest index) were analyzed to determine the most effective treatment. The results demonstrated that both IBA and GA independently modulated the expression of genes controlling the differentiation of the lignification layer (LL) and separation layer (SL), which are essential for pod opening. Overall, IBA treatments produced stronger positive effects on growth and yield indices compared with GA. Correlation analyses revealed that *FUL* and *RPL* had the highest positive associations with seed yield and harvest index, whereas *IND* exhibited negative correlations with thousand-seed weight and plant height. Considering the combined evidence from yield indices, quantitative trait analyses, pod dehiscence rates, and the expression patterns of key genes, IBA at 40 mg L^{-1} emerged as the most effective treatment for reducing seed shattering in flixweed. Plants treated with IBA 40 mg L^{-1} achieved the highest harvest index, showed elevated expression of the seed-retention genes *FUL* and *RPL*, and exhibited reduced expression of the shattering-promoting genes *IND* and *ALC*. Overall, IBA at 40 mg L^{-1} proved to be the most effective treatment in enhancing yield-related traits and reducing silique shattering. These findings suggest that the application of IBA at this concentration represents a practical and viable strategy to mitigate yield loss in flixweed cultivation. The strong edge between *HEC3* and *RPL*, along with their high CC values, suggests that these genes are suitable targets for expression manipulation or transient genetic interference (e.g., RNAi/CRISPRi) to further elucidate the regulatory mechanisms of seed shattering in flixweed. Taken together, the network topology analysis, highlighting *HEC3* and *RPL* as central regulatory nodes, aligns with the observed transcriptional modulation and physiological improvements under IBA treatment, collectively linking molecular coordination with reduced pod shattering and enhanced yield performance in flixweed.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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Generative AI statement

The author declares that generative AI (artificial intelligence) tools, including Grammarly & QuillBot, were used to enhance the language & clarity of this work. The author takes full responsibility for the accuracy & integrity of the content.

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