# Exploratory analysis of salicylic acid responsive long non coding RNAs linked to abiotic stress-related pathways in Datura metel

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**Research Article**

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# Exploratory analysis of salicylic acid responsive long non coding RNAs linked to abiotic stress-related pathways in *Datura metel*

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**ABSTRACT**

Background:

Long non-coding RNAs (lncRNAs) act as key regulators of plant responses to abiotic stresses, such as drought, cold, heat, and salinity. In parallel, the exogenous application of certain chemical compounds has been shown to enhance plant stress tolerance, potentially through lncRNA-mediated regulatory pathways. Salicylic acid (SA) is a phytohormone involved in the regulation of plant responses to both biotic and abiotic stresses. This study reanalysed publicly available RNA-seq data to identify and characterize SA-responsive lncRNAs associated with abiotic stress tolerance in *Datura metel*, a medicinally important plant with limited genomic resources.

Results:

A total of 65,469 transcripts were classified as putative lncRNAs from control and SA-treated leaf transcriptomes. Comparative expression analysis identified subsets of lncRNAs and protein-coding genes showing distinct expression patterns between treatments. Functional annotation of protein-coding genes associated with these expression changes showed significant enrichment in stress-related processes, including responses to drought, salt, osmotic stress, and oxidative stress. *Cis*- and *trans*-target prediction analyses suggested potential regulatory associations between SA-responsive lncRNAs and these genes, many of which were involved in glutathione metabolism, abscisic acid signalling pathways, protein serine/threonine kinase activity, inositol metabolism, and antioxidant biosynthesis.

Conclusions:

This study provides a genome-wide catalogue of lncRNAs and their associated expression patterns in *Datura metel* in response to SA treatment. The findings offer an exploratory view of the potential role of lncRNAs in enhancing the plant's tolerance to abiotic stresses. Although based on computational predictions and expression trends, these results highlight candidate stress-related lncRNAs that may serve as a valuable resource for future functional and experimental validation studies.

**Key Terms:** abiotic stress - chemical treatment - defense response - long non-coding RNA - stress tolerance

# 1 BACKGROUND

## 1.1 Long Non-Coding RNAs

Most of the eukaryotic transcriptome does not code for functional proteins. These are known as non-coding RNAs (ncRNAs), and some are produced in response to environmental factors or during particular stages of development (1). NcRNAs are often classified based on their length, with long non-coding RNAs (lncRNAs) typically defined as being longer than 200 nucleotides (2).

Initially, the presence of lncRNAs was considered as "transcriptional noise" and it was assumed that they lacked functional importance. However, more recent research has demonstrated that lncRNAs participate in a wide range of biological functions in both animals and plants (3). In eukaryotic cells, gene expression is regulated at multiple levels - epigenetic, transcriptional, post-transcriptional, translational, and post-translational. LncRNAs contribute significantly to regulation across many of these stages (4). In plants, lncRNAs are involved in regulating epigenetic modifications, flowering time, organ development, photomorphogenesis, and have increasingly been implicated in stress tolerance responses (5–8).

## 1.2 Abiotic Stress Tolerance

Plant growth, development, and productivity can be negatively affected by two main types of external stress: biotic and abiotic. Biotic stresses are caused by living organisms like bacteria and fungi, whereas abiotic stresses are the result of by environmental factors such as high temperatures, drought and excessive salinity (9). Since plants are stationary, they must adapt to such challenging conditions to ensure their survival. LncRNAs contribute significantly to the regulation of abiotic stress response pathways, including antioxidant activity, ROS homeostasis, MAPK signaling etc. in many plants (10).

## 1.3 Priming-Induced Acquired Stress Tolerance

Priming-induced acquired stress tolerance refers to a process where prior exposure to mild stress enhances a plant’s ability to withstand subsequent stresses compared to plants that haven’t experienced the initial stress (11). This prior exposure enables a faster and stronger stress tolerance response in the plant due to a kind of stress “memory”. The priming stimulus and the subsequent stress the plant is exposed to can be of the same type, known as *cis*-priming, or of different types, referred to as *trans*-priming or cross-stress tolerance (12). For instance,

in one study, seeds of chickpea (*C. arietinum* cv. Anuradha) and lentil (*Lens culinaris* cv. Ranjan) treated with sodium chloride exhibited enhanced tissue tolerance when later subjected to salt stress. This is an example of *cis*-priming (13). On the other hand, Bermuda grass (*Cynodon dactylon*) exposed to cold stress showed improved tolerance to salt stress, illustrating *trans*-priming (14).

### 1.4 Link between lncRNAs and Exogenous Chemical Application in Plants

Abiotic stress significantly limits plant growth and yield, prompting the development of various strategies to improve plant stress tolerance. One commonly utilized approach involves the external application of chemical compounds like methyl jasmonate, salicylic acid and abscisic acid, which mimic abiotic stress conditions and help activate the plant's defense mechanisms. Chemical priming of this nature is a type of the *trans*-priming described above (15). For example, treating Chinese crab apple (*Malus hupehensis*) with abscisic acid helped mitigate the harmful effects of excessive cadmium exposure (16). Likewise, applying melatonin externally to tomato (*Solanum lycopersicum*) under salt stress enhanced its tolerance by influencing several mechanisms, including the regulation of enzymes related to proline and carbohydrate metabolism in seedlings (17).

Both lncRNAs and exogenous chemical applications may play a role in plant stress response; therefore, the link between them is intriguing. Gene expression regulated by lncRNA-mediated pathways is a possible mechanism by which exogenous chemical application leads to stress tolerance (10).

For instance, in strawberry (*Fragaria* × *ananassa*), 412 lncRNAs were found to be differentially expressed following abscisic acid treatment, and many of these were linked to pathways involved in heat, drought, and osmotic stress responses (18). Another study on cassava (*Manihot esculenta*) examined how lncRNA expression changed in response to treatments with polyethylene glycol (which simulates drought) and melatonin (which promotes drought tolerance). Differentially expressed lncRNAs included 75 under polyethylene glycol treatment, 68 under melatonin treatment, and 42 under both treatments. Moreover, 28 lncRNA-mRNA pairs were found that potentially regulate nearby genes involved in light signalling, fatty acid metabolism, secondary metabolite production, and tetrapyrrole synthesis (19).

The possible link between lncRNAs and exogenous chemical treatments in the defense of crops and medicinal plants against abiotic stress remains poorly understood. This study aims to contribute to the growing body of knowledge in this emerging area.

### 1.5 *Datura metel* and Salicylic Acid

The Solanaceae family consists of approximately 90 genera and 3000–4000 species, which are widely used for food, medicinal and ornamental purposes. 14 of these species are part of the *Datura* genus. *Datura metel*, commonly known as 'Devil's Trumpet', is a perennial herbaceous plant belonging to this family (Figure 3). It is widely distributed in tropical regions with warm temperatures throughout Asia (20,21). *Datura metel* has a wide range of applications in traditional medicine, due to its anti-inflammatory, analgesic, antipyretic, antispasmodic, antitussive, anti-asthmatic, antimicrobial and antidiabetic properties. These properties are mostly a result of the production of potent secondary metabolites, such as tropane alkaloids (ex: hyoscyamine, scopolamine, anisodamine, anisodine etc.), flavonoids, saponins, steroids, tannins and withanaloids. Such metabolites when extracted and isolated have great value in the pharmaceutical industry (22–24).

Figure 1: *Datura metel* plants feature dark green, broadly ovate, shallowly lobed leaves and large, trumpet-shaped flowers with a sweet fragrance

Plants from the *Datura* genus, like all other plants, are susceptible to abiotic stresses. For instance, when *Datura metel* was exposed to high concentrations of zinc (heavy metal stress), growth and photosynthetic efficiency was significantly inhibited (25). Similarly, under low-temperature and high-temperature *Datura stramonium* root and shoot growth was impacted negatively (26).

Salicylic acid (SA) is a phenolic compound involved in the regulation of growth and development of plants, as well as important plant physiological processes. It also plays a major role in plant response to abiotic and biotic stresses. There is a large body of literature covering stress tolerance mechanisms induced by exogenous application of SA. These mechanisms include activation of antioxidant systems, production of secondary metabolites, optimization of mineral nutrient status and modulation of osmolytes synthesis. Additionally, many studies suggest SA is responsible for the transcriptional reprogramming that takes place in plants during stress responses (27,28). For example, drought stress conditions can severely affect the photosynthetic process in plants. However, SA-treated maize (*Zea mays*) plants that were deprived of water showed increased transcription of genes such as *psbA*, *RCA* and *rbcS* that prevented damage to photosynthetic organs, indicating enhanced stress tolerance (29).

LncRNA-mediated regulatory pathways may represent a mechanism through which exogenous SA treatments enhance stress tolerance in plants. In poplar (*Populus* × *euramericana*) leaves treated with SA, 49 lncRNAs associated with stress responses were differentially expressed. The genes targeted by these lncRNAs were primarily involved in MAPK signalling pathways, secondary metabolism, and hormone signalling. Among these, the gene *cytokinin dehydrogenase 1*—a target of lncRNA *MSTRG.27124.2*—is believed to contribute to drought stress resistance. Another gene, *fructose-diphosphate aldolase 1*, was regulated by seven different lncRNAs (*MSTRG.18764.2, MSTRG.24214.3, MSTRG.27124.2, MSTRG.3816.2, MSTRG.3931.1, MSTRG.5940.1,* and *MSTRG.929.1*). The activity of this enzyme has been linked to improved tolerance to both cold and salt stress (30).

There are a few studies that have looked at effects of SA application on *Datura metel*. For example, some studies suggests that SA treatment results in a significant increase in production of the alkaloids, hyoscyamine and scopolamine (31). However, this is contradicted by a paper that shows no significant change in the concentration of another alkaloid, atropine, following SA application (32). No studies have focused on the stress tolerance effect of SA on *Datura metel*.

Thus, there are prior findings suggesting that SA may serve as an effective chemical priming agent to enhance resilience to abiotic stresses in several other species of plants, potentially through lncRNA-mediated regulatory pathways. Building on this evidence, this study investigated whether exogenous SA treatment induced the expression of lncRNAs that regulated genes associated with abiotic stress tolerance in *Datura metel*, a medicinally

important plant species. Given the funding constraints typical for non-model plant research, this study generated high-depth RNA-seq libraries for each condition. Therefore, this study emphasizes QC-verified measurement quality, effect sizes, and single-sample pathway trends, while avoiding confirmatory gene-level claims.

## 2 METHODS

The workflow employed in this study was developed by integrating methodologies described across numerous published lncRNA studies (18,30,33).

### 2.1 RNA-Seq Data

The RNA sequencing data used in this study was from a previously published dataset (34). Total RNA was extracted from *Datura metel* L. leaf tissue under two conditions: untreated leaves and leaves treated with 5mM exogenous salicylic acid, and a *de novo* transcriptome assembly was conducted. RNA integrity and library metrics were assessed from QC reports provided by the authors of the study.

### 2.2 Prediction of lncRNAs from RNA-Seq Data

The coding probability of the *Datura metel* transcripts were predicted using CPAT software (v3.0.5) (35). Any transcripts recognized as non-coding that were shorter than 200 bp or encoded open reading frames (ORFs) longer than 100 amino acids were filtered out. PLEK software (v1.2) was also used to distinguish between lncRNAs and mRNAs of the transcriptome (36). Furthermore, TransDecoder software (v5.7.1) was used to identify and exclude any transcripts with potential coding regions (37). The intersection of transcripts filtered by CPAT, PLEK and TransDecoder were assigned as potential lncRNAs. Transcripts overlapping known protein domains (Pfam/InterPro) or structured RNAs (Rfam; tRNA/rRNA) were removed. Multi-exon status and minimum expression (TPM $\geq$1) were used to reduce false positives. Additionally, the transcripts with evidence of protein-coding potential identified from TransDecoder and PLEK were classified as mRNAs.

### 2.3 Identification of Differentially Expressed lncRNAs and mRNAs

Differential expression analysis was performed using the NOISeq (v3.21) package on all transcripts in a pairwise comparison without biological replicates (38). mRNAs and lncRNAs

with a probability ≥ 0.9 and log2 fold change ≥ 1 (up-regulated) or ≤ −1 (down-regulated) were considered differentially expressed (DE) and therefore SA-responsive. Because only a single biological library was available per condition, gene-level differential expression was treated as exploratory. Gene-level contrasts are reported as log2 fold changes and ranked NOISeq probabilities rather than confirmatory statistical tests. Where transcript quantification produced bootstrap or Gibbs inferential replicates, these were used to characterize technical measurement uncertainty; however, they do not substitute for biological replication. Due to the absence of replicates, formal false discovery rate (FDR) control was not applied at the gene level.

### 2.4 Gene Ontology and Pathway Enrichment Analysis

EggNOG-mapper tool (v2.1.13) was used to conduct functional annotation and obtain gene ontology (GO) labels and KEGG orthology (KO) identifiers and pathways for predicted mRNAs (39). This was followed by GO enrichment analysis carried out by the AgriGO web tool (v2.0), with the permutated false discovery rate value cut-off of 0.05 applied across all tested terms using the full expressed gene universe as background (40). KEGG pathway enrichment analysis was carried out using Kyoto Encyclopedia of Genes and Genomes (KEGG) database (41) and a hypergeometric test performed using the *clusterProfiler* R package (42). Fisher's exact test on the top 20 most common KO terms was also carried out through R for a count-based enrichment approach. However, KEGG pathway enrichment did not yield significant results.

### 2.5 *Cis*-Target Gene Prediction of SA-responsive DELs

Due to the absence of a chromosome-level reference genome assembly, cis relationships were inferred based on genomic proximity within the same assembled contig. Bedtools software (v2.31) was used to identify the protein-coding genes located within 100 kb upstream and downstream of differentially expressed lncRNAs (DELs) on the same contig (43). These neighbouring genes were then cross-referenced with the list of differentially expressed genes (DEGs) identified previously, and any overlap was designated as putative *cis*-regulatory targets of DELs.

### 2.6 *Trans*-Target Gene Prediction of SA-responsive DELs

LncTar software was used to identify DEGs that could potentially act as *trans*-targets of DELs (threshold: ndG ≤ -0.1) (44). The targets with potential stress tolerance related functions were

selected based on previously assigned GO labels. Cytoscape (v3.10.3) software was then used to build a network of the interactions between stress related *trans*-targets and DELs (45).

## 3 RESULTS

### 3.1 Genome-Wide Identification of mRNA and lncRNA in Leaves of *Datura metel* Treated with SA

Two strand-specific libraries from control group (clean_DL1) and the SA-treated group (clean_DSL1) were generated following RNA sequencing, containing 57,710,549 and 54,651,451 clean reads respectively. Libraries were prepared using TruSeq Stranded Total RNA with Ribo-Zero Plant. As the number of reads is over 50 million, the sequencing depth of this data is relatively high. The RNA integrity number (RIN) of both samples was above 8, suggesting high quality data appropriate for transcriptome analysis. The percentage of the GC content in the control group's leaves was slightly higher than in the SA-treated group. The value of the Q20 proportion for the samples was more than 98%, indicating that the RNA-seq data was highly reliable (Table 1, Figure 2). A total of 226,540 transcripts were obtained following sequence assembly. 20,492 transcripts were categorized as 'coding' and 206,048 transcripts were categorized as 'non-coding' or having no coding potential (Figure 3). This was filtered down to 65,469 potential lncRNAs meeting length and ORF criteria. (A full catalogue of putative lncRNAs is provided in Table S1 of Additional File 1.)

Table 1: Statistical data of RNA-seq reads from leaves treated with control and SA, showing that both libraries passed quality criteria

| Sample | Total Read Bases (bp) | Total Reads | GC% | AT% | Q20% | Q30% | RIN |
|---|---|---|---|---|---|---|---|
| clean_DL1 | 17,628,893,942 | 116,747,642 | 47.72 | 52.28 | 98.06 | 94.26 | 8.5 |
| clean_DSL1 | 16,676,673,144 | 110,441,544 | 45.17 | 54.83 | 98.18 | 93.99 | 8.6 |

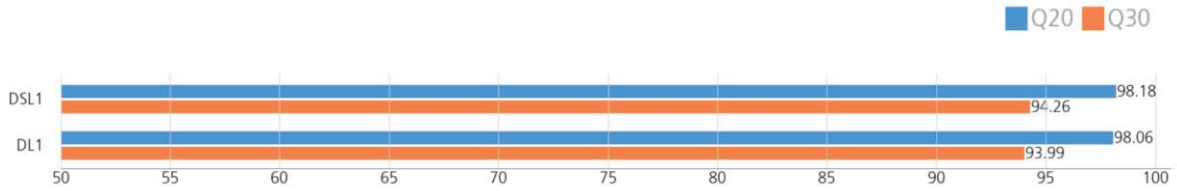



Figure 2: Q20/Q30 scores of raw data

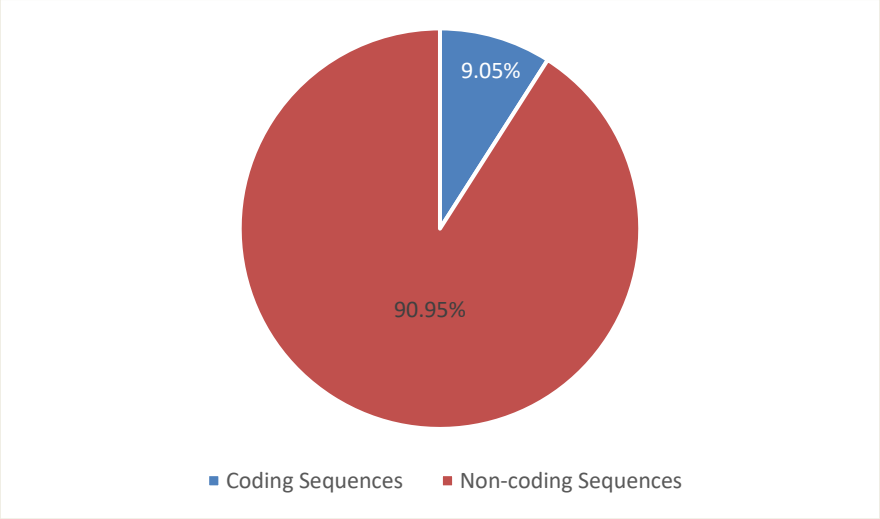



Figure 3: Percentage distribution of coding and non-coding transcripts

### 3.2 Identification of Differentially Expressed Genes and lncRNAs

By comparing the control group and the SA-treated group, 13,865 genes out of 20,492 were identified as being differentially expressed, with 6635 being upregulated and 7230 being downregulated. The high number of DEGs could be influenced by false positives due to the lack of replicates. Therefore, this paper focuses on effect size over probability to prioritize genes with substantial changes in expression, rather than solely relying on statistical significance. Similarly, a total of 310 DELs were identified. Of these, 150 were upregulated and 160 were downregulated (Figure 4). It is important to treat these results as exploratory and not as confirmed significance.

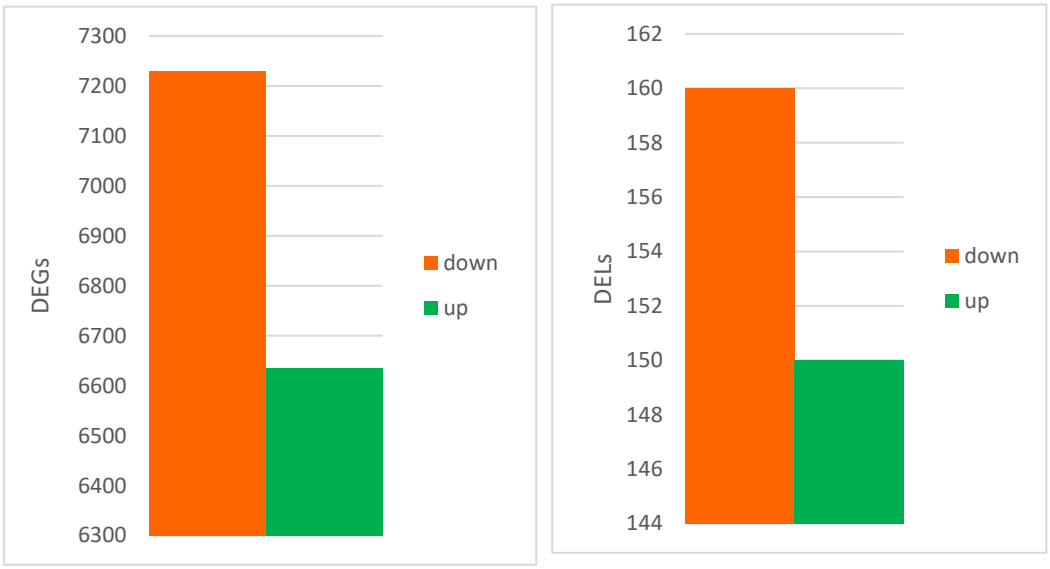
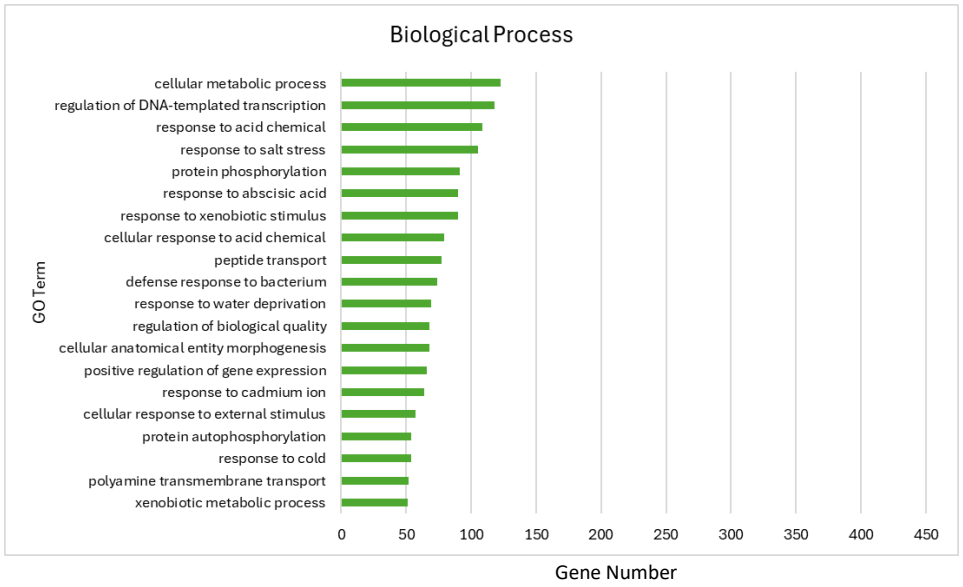
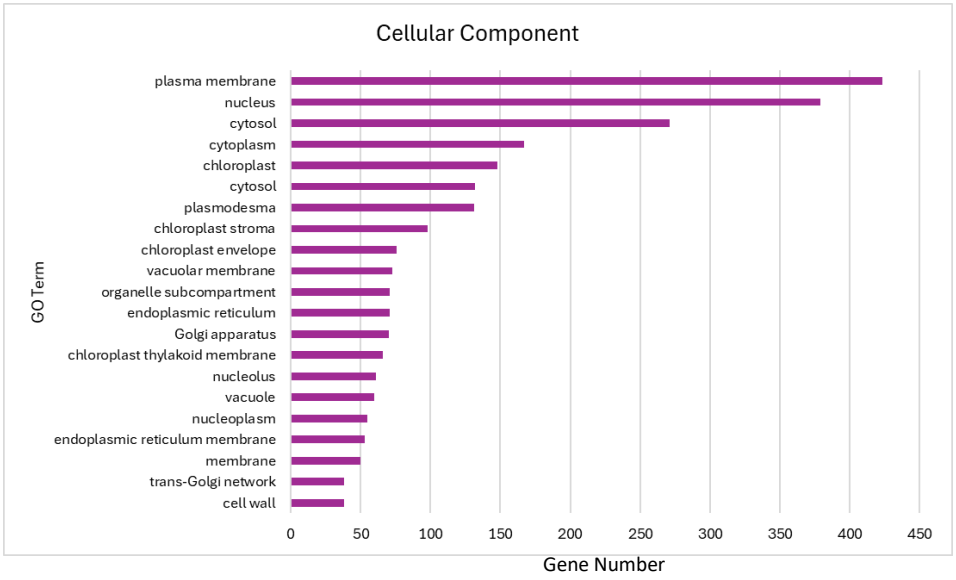



Figure 4: The number of DEGs and the number of DELs that have been upregulated and downregulated.

### 3.3 Functional Annotation and Enrichment Analysis of DEGs

GO annotation was carried out to classify DEGs into “biological process”, “cellular component”, and “molecular function” categories as seen in Figure 5. Among biological processes, over 100 genes were annotated as being involved in response to acid chemical. Other overrepresented GO terms included ‘response to salt stress’, ‘response to water deprivation’, ‘response to cadmium ion’ and ‘response to cold’. Additionally, over 50 genes were shown to be involved in ‘response to bacterium’.



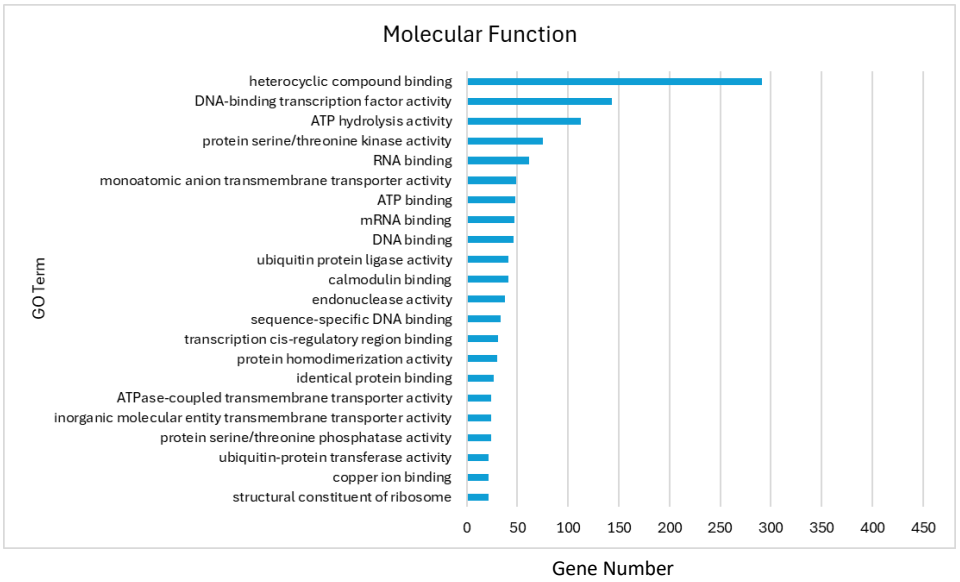



Figure 5: Top 20 GO annotations of DEGs for 3 main categories based on gene count

Enrichment analysis shows that 'response to acid chemical' is, as expected, the most enriched GO term (Figure 6). Other enriched terms include "protein serine/threonine phosphatase activity" and "glutamine family amino acid metabolic process". There is evidence that both these terms are linked to plant stress response.

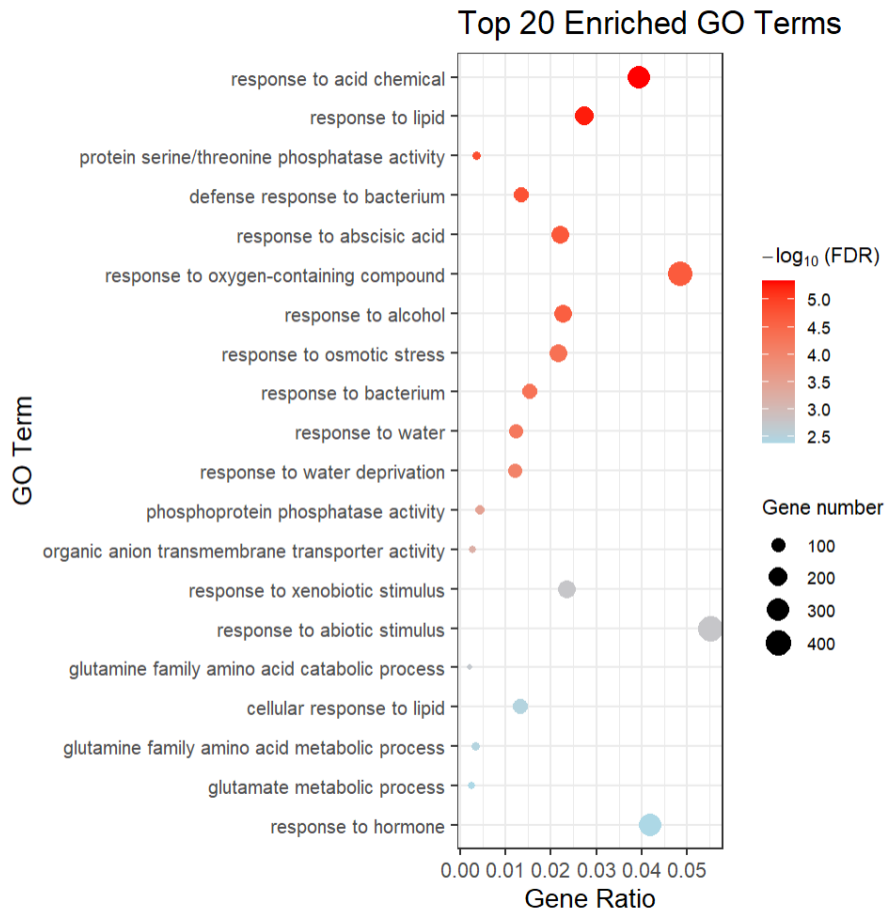



Figure 6: Top 20 Enriched GO Terms among DEGs

Enrichment analysis was performed to identify significantly overrepresented KEGG pathways among DEGs, as is the standard methodology. However, the over-representation tests, including both standard hypergeometric tests and count-based Fisher's exact tests, failed to detect any significantly enriched pathways after multiple testing correction. This lack of significant enrichment is likely due to the limited sample size (a single RNA-seq sample), which restricts statistical power. Furthermore, annotation resulted in only a few DEGs being confidently assigned to KEGG orthology (KO) terms, limiting the ability to detect pathway-level patterns. However, as can be seen in the discussion, certain pathways such as MAPK signaling and glutathione metabolism were considered in this study based on biological relevance. These pathways could be of interest in future studies, particularly with larger sample sizes that would provide greater statistical power.

### 3.4 Identification of *Cis*-Target Genes

*Cis*-acting lncRNAs function close to the site of transcription and typically influence the expression of adjacent genes, while *trans*-acting lncRNAs regulate distant genes. 372 DEGs were identified as being putative *cis*-targets for DELs. There were seven cis targets directly associated with stress responses (Figure 7). All seven target genes were annotated as responding to oxidative stress (Table 2). Detailed lists of all putative cis pairs, including genomic coordinates and binding scores, are available in Table S2 of Additional File 1.

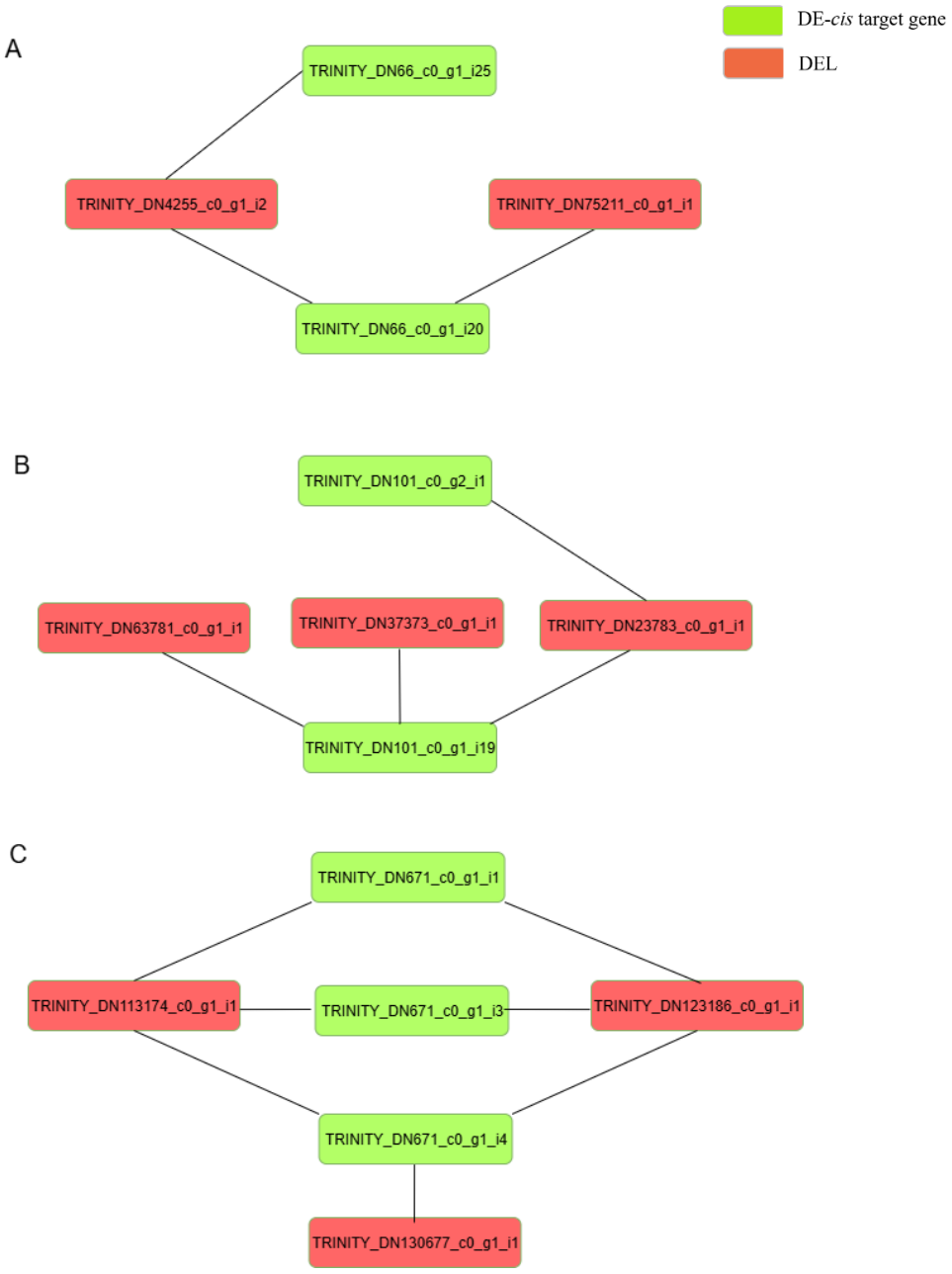



Figure 7: Interactions between DELs and DE-*cis* target genes related to stress response.

Table 2: Summary of GO annotations of stress related DEGs targeted by DELs in *cis*

| Target Gene | Responds To | Activity |
|---|---|---|
| *TRINITY_DN66_c0_g1_i20*<br>*TRINITY_DN66_c0_g1_i25* | ➢ oxidative stress<br>➢ jasmonic acid<br>➢ insect<br>➢ wounding | ➢ acid phosphatase activity<br>➢ transcription factor binding |
| *TRINITY_DN101_c0_g2_i1*<br>*TRINITY_DN101_c0_g1_i19* | ➢ oxidative stress<br>➢ water deprivation<br>➢ cold<br>➢ cytokinin<br>➢ red or far-red light<br>➢ karrikin | ➢ glutathione transferase activity<br>➢ 2,4,6-trinitrotoluene catabolic process<br>➢ cell communication<br>➢ peptide binding |
| *TRINITY_DN671_c0_g1_i1*<br>*TRINITY_DN671_c0_g1_i3*<br>*TRINITY_DN671_c0_g1_i4* | ➢ reactive oxygen species<br>➢ salt stress<br>➢ cytokinin | ➢ oxidoreductase activity<br>➢ 1-aminocyclopropane-1-carboxylate oxidase activity<br>➢ ethylene biosynthetic process |

### 3.5 *Trans*-Target Gene Identification

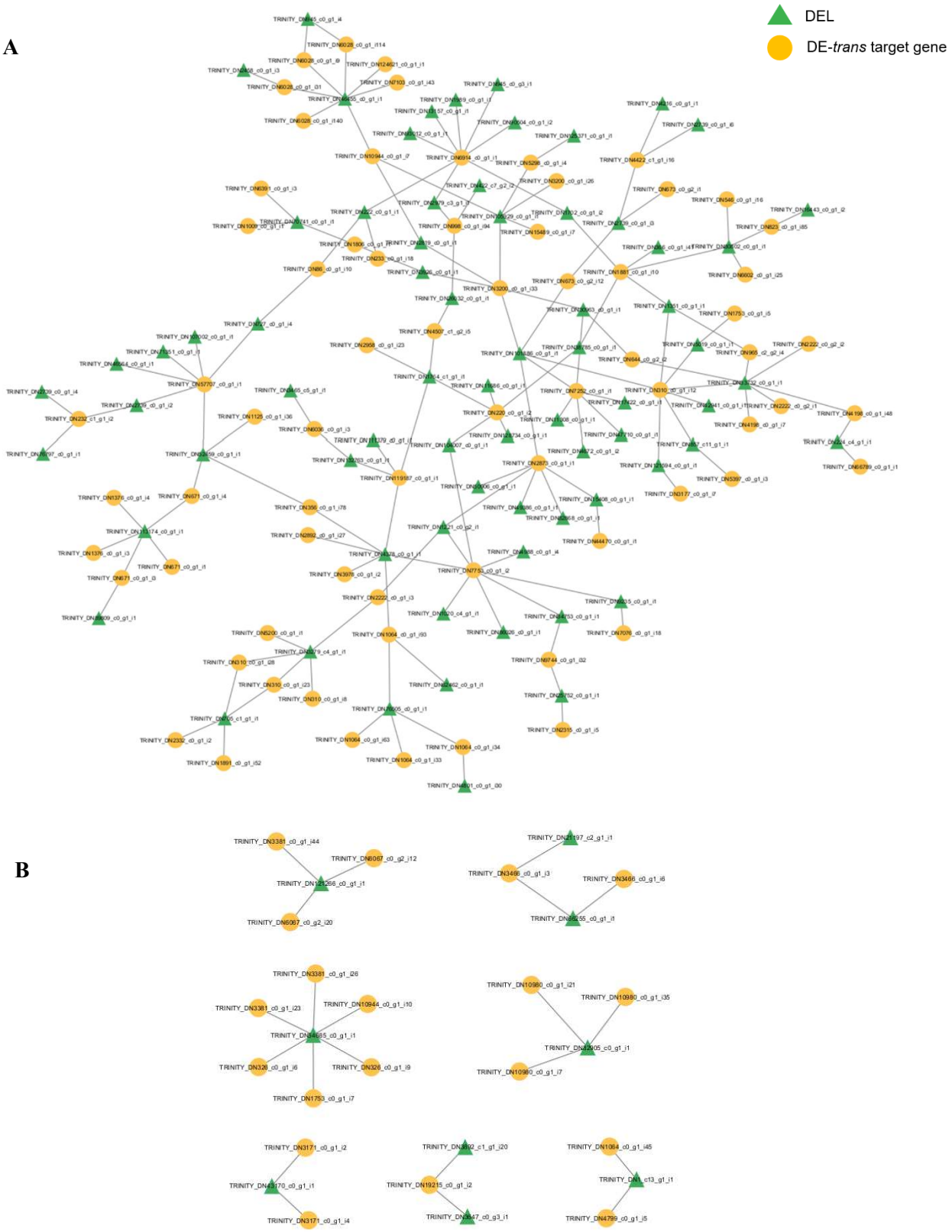



Figure 8: Networks of DELs and their stress response related DE-*trans* targets. An additional 12 DEGs were each individually targeted by 12 DELs (not pictured above).

101 DEGs were identified as stress response related *trans*-targets of 94 DELs (Figure 8). Some of these DEGs were targeted by several DELs, suggesting that they may be especially relevant in abiotic stress tolerance (Table 3). Detailed lists of all putative trans interactions, including binding scores, are available in Table S3 of Additional File 1.

Table 3: Stress related DEGs targeted by multiple DELs in *trans*

| Target Gene | Targeted By |
|---|---|
| *TRINITY_DN6914_c0_g1_i1*<br>*TRINITY_DN7753_c0_g1_i2* | 8 DELs |
| *TRINITY_DN310_c0_g1_i12* | 7 DELs |
| *TRINITY_DN57707_c0_g1_i1*<br>*TRINITY_DN2873_c0_g1_i1*<br>*TRINITY_DN7252_c0_g1_i1* | 6 DELs |
| *TRINITY_DN1881_c0_g1_i10*<br>*TRINITY_DN220_c0_g1_i2*<br>*TRINITY_DN3200_c0_g1_i33* | 5 DELs |

## 4 DISCUSSION

Given the single-replicate design, this exploratory study prioritizes effect sizes, consistent enrichment patterns, and concordance with prior literature over gene-level significance. Functional annotation revealed that DEGs in SA-treated leaves responded to acid chemical as well as several abiotic stresses (Figure 4). Enrichment analysis suggests that the terms “protein serine/threonine phosphatase activity” and “glutamine family amino acid metabolic process” are overrepresented (Figure 5).

Signal transduction is the process by which stress signals detected by the plant are transmitted through a cell as a series of events, ultimately resulting in the synthesis of stress-responsive transcription factors that can activate downstream effector genes. These pathways attempt to restore homeostasis in a cell that has been unbalanced by stressful conditions. Phosphatases are

important proteins that are involved in this process via phosphorylation and dephosphorylation. Serine/threonine-specific protein phosphatases are a specific family of phosphatases (46). In *Arabidopsis thaliana*, when one of these phosphatases (*OsPP108*) was overexpressed, high tolerance to salt, drought and osmotic stress was observed in adults (47). Another phosphatase (*AtPP5*) also forms complexes with heat shock proteins which enhance thermotolerance in *Arabidopsis* plants under heat stress (48). This aligns with the finding that serine/threonine phosphatase activity was enriched in SA-treated plants, suggesting SA may trigger similar protective phosphatase mechanisms in *Datura*.

The amino acids of the glutamine family - arginine, glutamate, glutamine and proline - may play a role in stress responses in plants. For example, many studies prove that proline and arginine accumulation occur in several plants under stress conditions. Glutamate is a precursor in proline synthesis (49). When SA was applied to lentil (*Lens esculenta*), proline metabolism was altered, and salt stress tolerance was enhanced (50). SA treatment may induce a similar response in *Datura* plants, which is consistent with the enrichment of the 'glutamine family amino acid metabolic process' term.

The terms “response to osmotic stress” and “response to water deprivation” are also enriched. Osmotic stress is caused by a sudden change in solute concentrations surrounding and inside the cell. It is mostly caused by water deficit (drought stress) or an accumulation of salts in the soil (salt stress). In this study, it appears that SA application resulted in *Datura metel* plant responding in a way that mainly mimics its response to drought stress and osmotic stress.

Interestingly, “defense response to bacterium” is also a highly enriched term. Though not the focus of this study, the negative impacts of biotic stresses caused by various pathogens can be mitigated by SA application. For instance, SA treatment of tomato (*Solanum lycopersicum*) induced stronger resistance and reduced the severity of symptoms caused by a pathogenic bacterium, *Xanthomonas campestris* pv. *Vesicatoria* (51).

### 4.1 *Cis*-Targets

#### 4.1.1 *TRINITY_DN66_c0_g1_i20* and *TRINITY_DN66_c0_g1_i25*

*TRINITY_DN66_c0_g1_i20* was targeted by 2 DELs, *TRINITY_DN4255_c0_g1_i2* and *TRINITY_DN75211_c0_g1_i11*. *TRINITY_DN66_c0_g1_i25* was also targeted by *TRINITY_DN4255_c0_g1_i2* (Figure 7A). Both these genes were upregulated and associated with acid phosphatase activity. An acid phosphatase gene known as *AtPAP15* was involved in

synthesis of myoinositol, which acted as a precursor to ascorbate biosynthesis in *Arabidopsis thaliana*. Transgenic lines of this plant with overexpression and inactivation of *AtPAP15* were generated to observe the phenotypic consequences of altering the gene being studied. There was a positive correlation between high expression levels of *AtPAP15* and high ascorbate levels as well as enhanced ozone and salt stress tolerance (Figure 9) (52). Ascorbate is a potent antioxidant found in most plant tissues. Reactive oxidative species (ROS) are by-products of aerobic metabolism that play significant roles in plant growth and development but can also be toxic if an excess is accumulated. Therefore, a balance must be maintained in between ROS production and ROS scavenging. Different abiotic stresses such as drought stress, salt stress etc. may upset this balance, resulting in oxidative stress as secondary stress (53). Ascorbate can eliminate ROS and make a plant more tolerant of such conditions. A study on effects of heat and cold stress on *Datura stramonium* showed that concentrations of ascorbic acid along with several other enzymatic antioxidants increased as a protective effect against oxidative damage (26). Both *TRINITY_DN66_c0_g1_i20* and *TRINITY_DN66_c0_g1_i25* were annotated as being involved in "response to oxidative stress", suggesting a possible mechanism where SA-induced lncRNAs could enhance stress tolerance by elevating ascorbate production in *Datura metel*. Another study showed that in *Arabidopsis thaliana* as well as *Glycine max* (soybean), overexpression of an acid phosphatase gene known as *GmHAD1* was shown to increase the efficiency of phosphate utilization and thereby increase tolerance to phosphorus deficiency stress (Figure 9) (54).

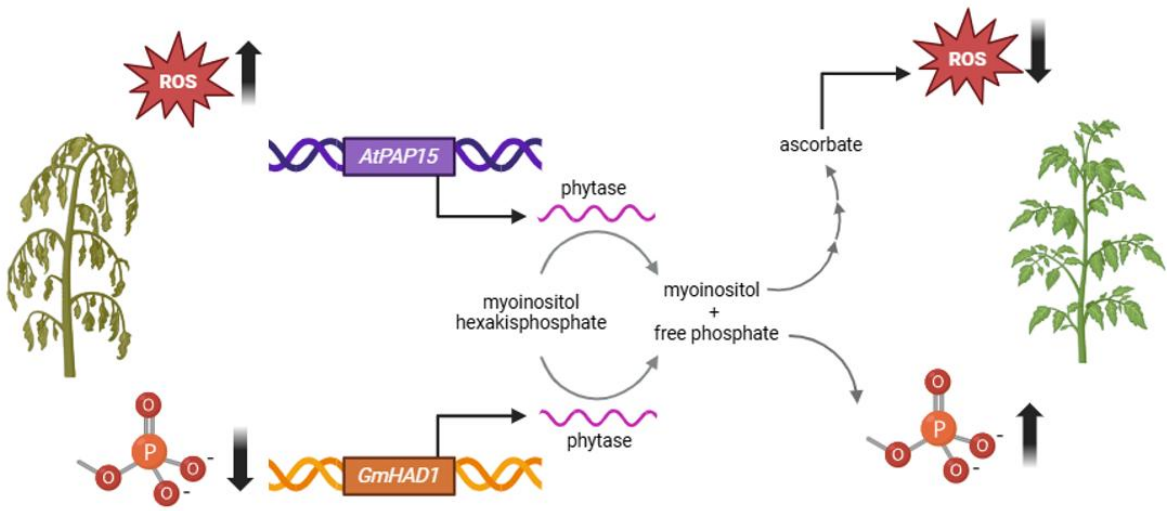



Figure 9: Mechanism of two acid phosphatase genes in *Arabidopsis* that combat oxidative and phosphate deficiency stress

Interestingly, these target genes also showed a response to wounding and insects. This can be explained by the acid phosphatase activity as well. In *Arabidopsis thaliana*, *Arabidopsis* vegetative storage protein 2 (*AtVSP2*) was overexpressed following wounding or insect feeding. The acid phosphatase activity of accumulated *AtVSP2* was able to reduce the plant's susceptibility to insect attacks (55).

Both target genes are also associated with "transcription factor binding" and "response to jasmonic acid". The APETALA 2/ETHYLENE-RESPONSIVE FACTOR (*AP2/ERF*) transcription factor family plays a major role in stress response in several plants. Abscisic acid (ABA) levels also increase in response to oxidative stress. In *Arabidopsis thaliana*, increased ABA levels activated SNF1-related kinase 2 (*SnRK2*), which are serine/threonine kinases. *SnRK2*, in turn, phosphorylated and activated ABRE-BINDING PROTEIN/FACTOR (*AREB/ABF*) (56). *AREB/ABF*s were able to bind to the ABA-responsive element (*ABRE*), which is a promoter sequence found upstream of gene that encodes for a *AP2/ERF* transcription factor known as DRE-BINDING PROTEIN 2A (*DREB2A*). *DREB2A* improved tolerance to drought when overexpressed by activating the expression of many stress-inducible genes (Figure 10) (57,58). Similarly, in transgenic *Arabidopsis* with deficient DRE-BINDING PROTEIN 1 (*DREB1*s), the plants were very sensitive to cold stress. This is because in wild type plants, *DREB1*s regulated 414 cold responsive genes, contributing to freezing tolerance (59). Another *AP2/ERF* transcription factor known as octadecanoid-responsive AP2/ERF-domain transcription factor 47 (*ORA47*) targeted genes involved in jasmonic acid (JA) biosynthesis under water stress conditions (60). JA is a plant growth regulator that enhances the antioxidant activity of the plant in response to abiotic stresses such as heat, cold, drought, heavy metal etc. (61). SA-responsive lncRNAs in *Datura metel* may modulate drought and oxidative stress tolerance by influencing ABA- and JA-mediated transcription factor related pathways, similar to *AP2/ERF* regulatory network.

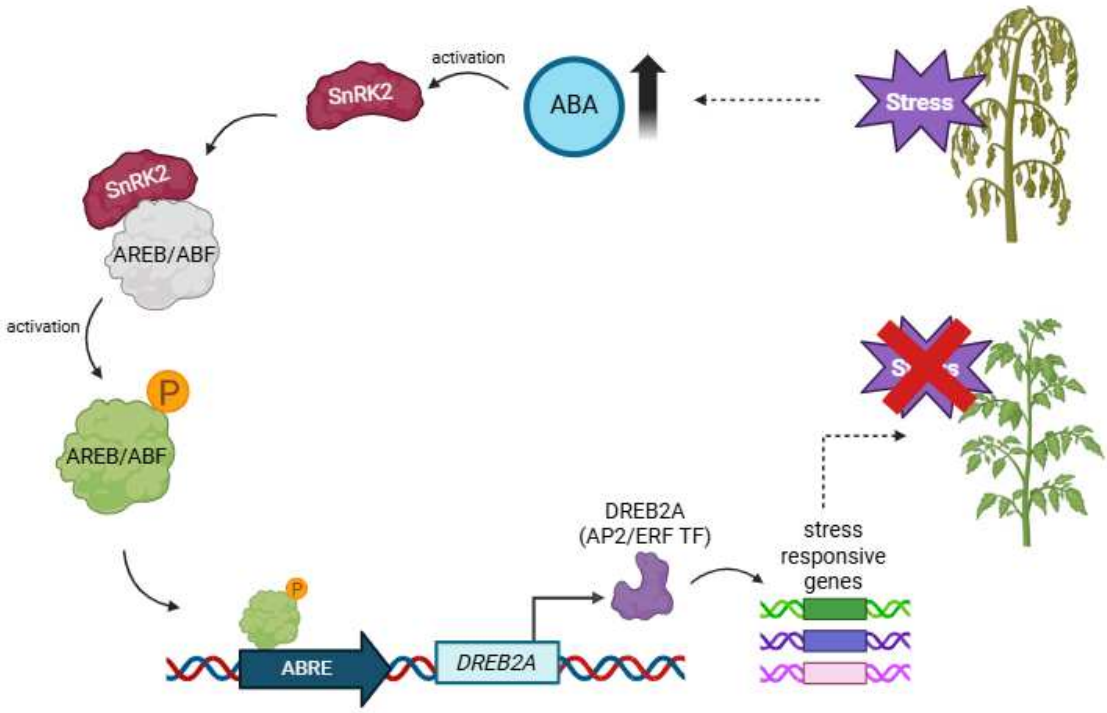



Figure 10: How stress conditions trigger AP2/ERF transcription factors to carry out stress tolerance functions in *Arabidopsis*

The KEGG pathway linked with these target genes is the MAPK signalling pathway. Mitogen activated protein kinases (MAPKs) are a major component in plant signalling that can be activated by abiotic stress conditions. MAPKs then target a variety of transcription factors and effector genes in a cascade reaction, ushering in an appropriate stress response. For example, in *Arabidopsis thaliana* under salt stress conditions, levels of phosphatidic acid (PA) were raised. PA, a lipid messenger, interacted with MAPKs MKK7 and MKK9, which further phosphorylated the MAPK MKK6. MKK6 then phosphorylated SALT OVERLY SENSITIVE 1 (*SOS1*), a membrane protein that actively transports $Na^+$ out of the cell to maintain homeostasis and combat effects of excessive salinity (Figure 11) (62).

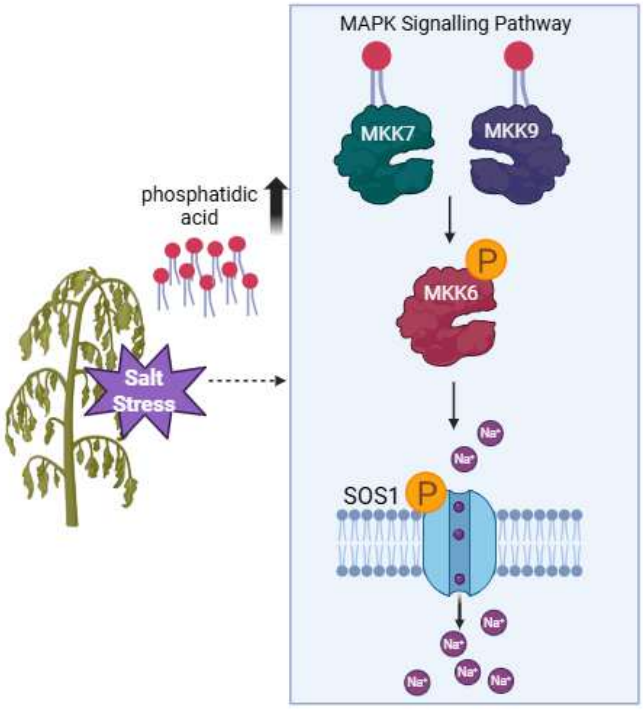



Figure 11: Involvement of MAPK signalling pathway in salt stress tolerance in *Arabidopsis*

4.1.2 *TRINITY_DN101_c0_g1_i19* and *TRINITY_DN101_c0_g2_i1*

*TRINITY_DN101_c0_g1_i19*, a glutathione s-transferase (*GSTU1*) gene, which responds to cold, heavy metal and drought stress, is targeted by 3 DELs and upregulated. One of these DELs also targets another *GSTU1*, *TRINITY_DN101_c0_g2_i1* (Figure 7B). Glutathione transferases (*GST*s) have a direct role in maintaining the balance of the cellular redox state and protecting plants from oxidative stress (63). A study similar to this one studied the effects of exogenously applied salicylic acid on tomato (*Solanum lycopersicum*) and found that *GST* genes were upregulated and in turn, salt stress tolerance was increased (64).

These genes are also involved in 2,4,6-trinitrotoluene (TNT) catabolism according to their GO annotations. TNT is a toxic chemical with mutagenic properties and the ability to induce oxidative stress by generating ROS (65). In *Arabidopsis thaliana*, overexpressed *GST*s led to the ability to detoxify TNT by degrading it into TNT-gluta-thionyl products, allowing the plant to withstand oxidative stress conditions (66). An intriguing study suggests that the plants *Datura innoxia* and *Lycopersicon peruvianum* can be used in the bioremediation of TNT-contaminated soil, as they were able to break down the toxin into non-toxic aldehyde and carboxylic acid metabolites (67). The KEGG pathway associated with these target genes are,

as expected, ‘glutathione metabolism’, along with ‘metabolism of xenobiotics by cytochrome P450’.

*TRINITY_DN101_c0_g1_i19* and *TRINITY_DN101_c0_g2_i1* are also involved in regulation of response to red or far red light. Tomato (*Solanum lycopersicum*) grown under low red light:far red light ratio were better able to withstand salinity stress by upregulating genes that produced ROS-scavenging enzymes, such as superoxide dismutase, peroxidase, catalase and ascorbate peroxidase (68).

Another GO annotation suggests these genes respond to karrikin. Karrikins (KAR) are butanolide compounds that can be found in the smoke of burning vegetation (69). KARs bind to *KARRIKIN INSENSITIVE2* (*KAI2*) receptors. In *Arabidopsis thaliana*, *KAI2*-mediated signalling regulate genes with roles in cuticle formation, stomatal closure, anthocyanin biosynthesis and membrane integrity to enhance drought stress tolerance (70). Another study conducted on the same plant found that specimens with a defect in *KAI2* were more severely impacted by salinity stress, suggesting this pathway has an important role in mitigating negative impacts from such conditions (71).

There are several similar communication networks in plants controlled by signalling peptides that play roles in stress responses. For instance, peptides such as *CLE*, *IDA*, *PSK* and *CEP5* are involved in mitigating drought stress in several plants (72). Thus, the GO terms of ‘cell communication’ and ‘peptide binding’ in *TRINITY_DN101_c0_g1_i19* and *TRINITY_DN101_c0_g2_i1* can be explained.

Collectively, the targeting of these GST-associated genes in *Datura metel* by SA-responsive lncRNAs may suggest a coordinated regulatory role for lncRNAs in enhancing oxidative stress tolerance and environmental resilience through redox homeostasis, detoxification pathways, and stress-related signalling networks.

4.1.3 *TRINITY_DN671_c0_g1_i1, TRINITY_DN671_c0_g1_i3*, *TRINITY_DN671_c0_g1_i4*

*TRINITY_DN671_c0_g1_i1, TRINITY_DN671_c0_g1_i3* and *TRINITY_DN671_c0_g1_i4,* which respond to salt stress, are targeted by a pair of DELs, *TRINITY_DN113174_c0_g1_i1* and *TRINITY_DN1123186_c0_g1_i1*. *TRINITY_DN671_c0_g1_i4* is also targeted by *TRINITY_DN130677_c0_g1_i4* (Figure 7C). All three of these genes belong to the iron ascorbate-dependent oxidoreductase family.

Oxidoreductases have been proven to influence the mitigation of oxidative stress impacts. For example, in *Glycine max* (soybean), an oxidoreductase called *GmOXR17* interacts with overexpressed nuclear transport factor *GmNTF2B-1* and promotes ROS scavenging, thus enhancing drought stress tolerance (73). Similarly, another study showed that in two drought-tolerant genotypes of barley (*Hordeum vulgare*) under drought stress, genes coding for ascorbate-dependent oxidoreductases were upregulated when compared to a drought-sensitive genotype (74).

These target genes were found to have 1-aminocyclopropane-1-carboxylate oxidase (ACO) activity. They also 'respond to nitric oxide (NO)' and are involved in the 'ethylene biosynthetic process'. ACO regulates the production of ethylene, which is a phytohormone with a significant role in plant growth and development. However, some papers suggest that under stressful conditions, increased ethylene concentrations may have harmful effects on the plant. Interestingly, whether ACO genes are upregulated or downregulated seems to differ based on the species of plant and the type of stress they are exposed to. The exact role of ethylene in stress tolerance remains similarly unclear (75). Studies have suggested that NO and ACO production is synergetic. NO promotes increased activity of ACO via transcriptional activation and protein *S*-nitrosylation in tomato (*Solanum lycopersicum*) under salt stress. In this particular case, ethylene levels are elevated, and salinity tolerance is enhanced (76). A similar mechanism could have occurred in this *Datura metel* plant. In fact, increased ethylene levels could explain the 'positive regulation of seed germination' function in these target genes. In another study, transgenic *Arabidopsis thaliana* plants with overexpressed ACO genes were significantly more tolerant to flood stress than wild type plants (77). The KEGG pathway associated with these target genes is 'cysteine and methionine metabolism', which can potentially be attributed to ethylene being synthesized from methionine.

*TRINITY_DN671_c0_g1_i1, TRINITY_DN671_c0_g1_i3* and *TRINITY_DN671_c0_g1_i4* further responded to cytokinin, which plays a role in plant defence signalling pathways. The levels of this phytohormone often decrease in stress conditions as they have an inhibitory effect on the stress responsive genes. Interestingly, cytokinin works antagonistically with ABA, whose concentration increases with stress (Figure 12). Cytokinin-deficient transgenic *Arabidopsis thaliana* plants were shown to be significantly more tolerant to drought and salt stresses (78).

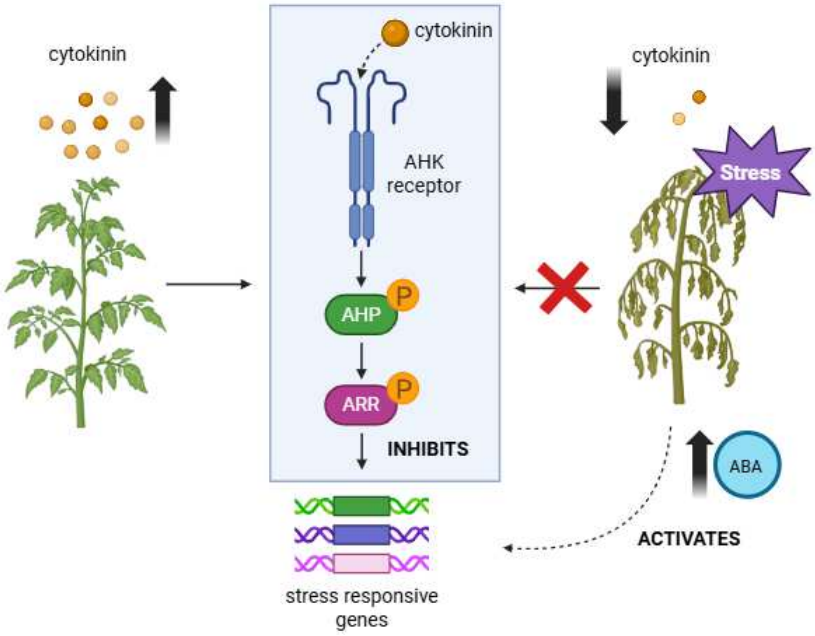



Figure 12: Cytokinins play different roles depending on whether the plant is undergoing stress conditions

Overall, the association of these oxidoreductase and ethylene-related genes with multiple stress-response pathways suggests that SA-responsive lncRNAs in *Datura metel* may contribute to salinity and drought tolerance by modulating redox homeostasis and hormone-mediated signalling.

These target genes were also associated with "response to fungus". Although this study focuses on stresses caused by environmental factors, there is some overlap between responses to biotic stresses and abiotic stresses. For example, upregulation of oxidoreductases in *Cucurbita pepo* (pumpkin) plants played a role in their ability to resist powdery mildew disease caused by the fungus *Podosphaera xanthii* (79).

There are a few genes targeted by the DELs that are not differentially expressed, but GO annotation indicates they would have similar functions to the targeted DEGs discussed above (Table 4). It is possible they were expressed alongside the target DEGs via lncRNA-mediated pathways.

Based on these results, it can be inferred that most DELs in *Datura metel* regulate target DEGs involved in mitigating oxidative stress, a common secondary effect of several primary abiotic stressors such as heat, cold, drought etc. This suggests that exogenous SA treatment may serve

as an effective chemical priming strategy by enhancing the plant's ability to cope with a broad range of abiotic stresses.

Table 4: Genes targeted by DELs in *cis* that are not differentially expressed and their probable functions

| | Annotated GO Terms<br>[P: Biological Process, F: Molecular Function] |
|---|---|
| DEL:<br>TRINITY_DN4255_c0_g1_i2<br>TRINITY_DN75211_c0_g1_i1<br><br>Cis-Target Gene:<br>TRINITY_DN66_c0_g1_i5 | P: response to acid chemical<br>P: response to oxidative stress<br>F: acid phosphatase activity<br>P: dephosphorylation<br>P: defense response to insect<br>P: response to jasmonic acid<br>F: transcription factor binding<br>P: response to insect<br>P: response to wounding<br>P: response to copper ion |
| DEL:<br>TRINITY_DN63781_c0_g1_i1<br>TRINITY_DN23783_c0_g1_i1<br><br>Cis-Target Gene:<br>TRINITY_DN101_c0_g1_i4 | P: response to cytokinin<br>P: response to cadmium ion<br>P: cellular response to external stimulus<br>P: regulation of response to red or far red light<br>P: response to oxidative stress<br>F: enzyme binding<br>P: response to Karrikin<br>F: glutathione binding<br>P: response to herbicide<br>P: 2,4,6-trinitrotoluene catabolic process<br>P: glutathione metabolic process<br>P: cellular response to water deprivation<br>P: cell communication<br>P: toxin catabolic process<br>F: glutathione transferase activity<br>F: peptide binding<br>P: response to cold |
| DEL:<br>TRINITY_DN23783_c0_g1_i1<br><br>Cis-Target Gene:<br>TRINITY_DN101_c0_g1_i12 | P: response to cytokinin<br>P: response to cadmium ion<br>P: peptide metabolic process<br>P: cellular response to external stimulus<br>P: regulation of response to red or far red light<br>P: response to oxidative stress<br>F: enzyme binding<br>P: response to Karrikin<br>F: glutathione binding<br>P: response to herbicide<br>P: 2,4,6-trinitrotoluene catabolic process<br>P: glutathione metabolic process<br>P: cellular response to water deprivation |

| | |
|---|---|
| | P: cell communication<br>P: toxin catabolic process<br>F: glutathione transferase activity<br>F: peptide binding<br>P: response to cold |

### 4.2 *Trans*-Targets

Several DEGs were targeted by multiple DELs in *trans* (Figure 8). TRINITY*_DN6914_c0_g1_i1* and *TRINITY_DN7753_c0_g1_i2* are both targeted by 8 DELs and involved in salt stress response. *TRINITY_DN7753_c0_g1_i2* responds to UV-B, which is a short wavelength radiation found in natural sunlight. This finding echoes results in *Pohlia nutans* (Antarctic moss) where under high levels of UV-B, 1459 lncRNAs were differentially expressed. These lncRNAs mainly targeted genes involved in the biosynthesis of flavonoids, which are secondary metabolites that are involved in abiotic stress tolerance responses (80). *TRINITY_DN7753_c0_g1_i2* also responds to hydrogen peroxide and has MAPK activity. As mentioned previously, ROS like $H_2O_2$ are generated in plants under abiotic stress conditions, resulting in oxidative stress. $H_2O_2$ acts as central signalling molecule in the subsequent stress tolerance mechanisms by activating MAPK cascades and even modulating expression of genes coding for antioxidant enzymes (81).

*TRINITY_DN310_c0_g1_i12*, which is targeted by 7 DELs, has a similar response to UV-B and MAPK activity as *TRINITY_DN7753_c0_g1_i2*. Additionally, it also responds to oxidative, osmotic and cold stress. GO annotation also suggests that there is a 'camalexin biosynthetic process' associated with this gene. However, camalexin accumulation is more closely linked to biotic stress tolerance than abiotic (82).

*TRINITY_DN57707_c0_g1_i1* is targeted by 6 DELs, responds to salt and drought stresses and expresses pyrophosphatase activity as well as ATPase-coupled transmembrane transporter activity. In addition to this, the 'establishment or maintenance of transmembrane electrochemical gradient' is an enriched biological process associated with *TRINITY_DN57707_c0_g1_i1.* Thus, the pyrophosphatase and ATP-ase in this case are likely vacuolar proton pumps, both of which can play a role in salt and drought stress tolerance (83). In *Arabidopsis thaliana*, plants overexpressing the *AVP1* gene that encodes a proton-pumping pyrophosphatase were shown to be more tolerant of salt and drought stresses than wild type plants (84).

The targeting of these stress-responsive genes by multiple DELs in trans suggests that SA-induced lncRNAs in *Datura metel* may coordinate ROS–MAPK signalling, UV-B responses, and ion homeostasis to enhance tolerance to salt, drought, and oxidative stress.

*TRINITY_DN2873_c0_g1_i1* and *TRINITY_DN7252_c0_g1_i1* are also each targeted by 6 DELs. The former responds to ozone and salt stress and has protein serine/threonine kinase activity. The latter responds to oxidative stress and is involved in glutathione metabolism and transcription factor binding. *TRINITY_DN7252_c0_g1_i1* also encodes a glutathione peroxidase, an enzyme that directly scavenges hydrogen peroxide(85). Multiple lncRNAs targeting this gene implies lncRNA regulation could reinforce ROS detoxification pathways (consistent with the GO term of "hydrogen peroxide catabolic activity" related to this gene)

*TRINITY_DN220_c0_g1_i2* and *TRINITY_DN3200_c0_g1_i33*, each targeted by 5 DELs, respond to salt and osmotic stress, respectively. *TRINITY_DN220_c0_g1_i2* has a role in the biosynthesis and degradation of lignin. Lignin content in plants is impacted by abiotic stresses. For instance, in transgenic *Arabidopsis thaliana* plants overexpressing *PeLAC10* gene resulted in increased lignin synthesis and consequently, increased drought stress tolerance (86). A similar lignification mechanism that promotes drought and salt stress tolerance can be observed in rice (*Oryza sativa*) and sweet potato (*Ipomoea batatas*) respectively (87,88).

*TRINITY_DN356_c0_g1_i78, TRINITY_DN965_c2_g2_i4, TRINITY_DN3200_c0_g1_i26, TRINITY_DN3200_c0_g1_i33* and *TRINITY_DN7252_c0_g1_i1* are *trans*-targets that could potentially be involved in inositol metabolic processes, according to their GO annotations. Inositol and its derivatives as well as relevant enzymes form an intricate network that have been proven to have a role in abiotic stress tolerance in plants such *Arabidopsis thaliana*, rice (*Oryza sativa*) and potato (*Solanum tuberosum*) (89). Inositol biosynthesis requires sufficient quantities of L-myo-inositol 1-phosphate synthase (MIPS). Overexpression of *TaMIPS2* genes resulted in increased inositol content and in turn, enhanced heat, salt, drought and cold stress tolerance in transgenic *Arabidopsis thaliana* plants (90). The DEG *TRINITY_DN3200_c0_g1_i33* has inositol-1,3,4-trisphosphate 5/6-kinase (ITPK) activity. ITPK also has a significant role in inositol metabolism (91). Thus, the trans-targets include genes that strengthen physical stress barriers (lignification) and osmoprotectant pathways (inositol biosynthesis), hinting that lncRNAs induced by SA could orchestrate both biochemical and structural stress tolerance mechanisms in *Datura*.

Many of the trans-targets also respond to ABA. ABA is known to initiate signal transduction in response to stressful conditions. It can do this in many ways, such as by binding to *PYR/PYL/RCAR* receptors. A study showed that *PYL5* receptors were overexpressed in rice (*Oryza sativa*) under drought stress conditions, resulting in increased ABA signalling and a stronger defence against water deprivation (92).

Some of the DEGs are only targeted by 1-2 DELs but still may play significant roles. For example, *TRINITY_DN356_c0_g1_i78* belongs to the class-III pyridoxal-phosphate-dependent aminotransferase family. Alanine aminotransferase (AlaAT), which catalyses the conversion of alanine and 2–oxoglutarate into pyruvate and glutamate, is an example of this type of enzyme. In soybean (*Glycine max*), waterlogging stress induced increased levels of AlaAT, suggesting a role in stress tolerance response (93). This gene was also involved in gamma-aminobutyric acid (GABA) catabolic process. GABA tends to accumulate in plants, and GABA metabolism is upregulated under stress conditions. In *Arabidopsis thaliana*, GABA is encoded by *Pollen-Pistil Incompatibility 2* (*POP2*) gene. A study depicted that mutant plants with loss of function in *POP2* gene were significantly more sensitive to salt stress damage than plants with functioning *POP2* (94). Another example is *TRINITY_DN6391_c0_g1_i3,* which may function as a glucan endo-1,3-beta-D-glucosidase. A study showed that in wheat (*Triticum aestivum*), a drought stress tolerant genotype had a higher accumulation of this enzyme when compared to a genotype more sensitive to drought stress. This may potentially be due to its role in cell wall biogenesis (95). Collectively, these results suggest that SA-induced lncRNAs and their associated DEGs may help coordinate metabolic and signalling pathways—such as GABA metabolism, alanine aminotransferase activity, and cell wall remodelling—to enhance *Datura metel*'s tolerance to drought, waterlogging, and salinity stress.

In summary, the trans-target analysis reveals several stress-response genes each associated with multiple lncRNAs. These genes span ROS signaling, osmoprotection, ion transport, and hormone-related pathways. Other trans-targets from Figure 7 have stress tolerance functions such as protein serine/threonine kinase activity, proline metabolism, acid phosphatase activity, transcription factor binding, jasmonic acid and cytokinin-mediated signalling described previously. This strongly indicates that the lncRNAs identified in SA-treated *Datura metel* are non-randomly targeting key stress adaptation nodes, reinforcing the notion that lncRNAs could play orchestrating roles in SA-mediated stress priming.

### 4.3 Limitations and Future Perspectives

The principal limitation in this study is the use of only a single sample without biological replicates for analysis. The absence of replicates reduces statistical power, limits the ability to assess variability, and increases the risk of false positives in differential expression analysis, making it difficult to draw robust conclusions about gene expression patterns. However, the application of stringent filters based on transcript length, ORF content, and known protein domains yielded high-confidence lncRNAs, many of which exhibit biologically coherent targeting of stress-related genes, reducing the likelihood that they represent false positives. Additionally, some of these limitations may be offset by the high sequencing depth of the dataset used. Nonetheless, all gene-level interpretations are presented as putative.

Another key limitation is the reliance on a de novo genome assembly in the absence of a reference genome for *Datura metel.* Therefore, this study depends heavily on homology-based methods for gene prediction and functional annotation. This can result in incomplete or inaccurate Gene Ontology (GO) and KEGG pathway analyses due to poor matches to known sequences, particularly for novel or species-specific genes. There is also a higher chance of missing annotations because of partial or fragmented genes caused by assembly errors or sequencing limitations. Additionally, functional enrichment analyses may be biased toward well-conserved genes with existing annotations, potentially overlooking biologically relevant but uncharacterized features.

The selected effects theory of function states that the function of a trait is the function for which the trait was naturally selected; meaning that a true function should have an evolutionary context (96). Since most lncRNAs exhibit low sequence conservation, proving functionality is challenging (97). This study, as well as most of the studies cited in this paper, proposes that an increased expression of lncRNAs under abiotic stress implies that lncRNAs have a role to play in stress tolerance. In these experiments, GO and KEGG analyses were performed to predict the functions of genes potentially targeted by lncRNAs. However, while such studies are useful for discovering new lncRNAs, they don’t provide definitive proof that lncRNAs directly contribute to abiotic stress responses - the observed relationships may simply be correlations (98).

Some studies in this area use transgenic plant lines modified through CRISPR to either knock out or overexpress specific lncRNAs. This approach enables researchers to observe resulting phenotypic changes, offering stronger evidence of lncRNA functionality (2). As such, more

research using these methods is necessary. Currently, only a small number of plant lncRNAs have clearly established biological roles with well-defined molecular mechanisms. As the field progresses, it's crucial to determine the exact functions of other lncRNAs as well. A key step forward will be to design biochemical experiments that rely less on computational predictions and more on direct experimental evidence to validate the findings of prior studies.

Regarding chemically induced abiotic stress tolerance, the limited existing research has yet to determine whether lncRNAs mediate the effects of priming, or if other mechanisms are responsible. Still, the observed increase in lncRNA expression under such conditions is intriguing and deserves further investigation.

## 5 CONCLUSIONS

This study provides the first transcriptomic insight into how exogenous salicylic acid may prime *Datura metel* for abiotic stress tolerance, with a novel focus on the regulatory roles of lncRNAs. A total of 13,865 differentially expressed genes and 310 differentially expressed lncRNAs were identified, suggesting that significant transcriptomic reprogramming is induced by SA application. Functional annotation highlighted key biological processes such as responses to acid chemicals, osmotic stress, drought, and oxidative stress - confirming the role of SA in enhancing abiotic stress tolerance.

Several DELs were predicted to regulate stress-related genes in *cis*, including those involved in glutathione metabolism, MAPK signaling, and antioxidant biosynthesis, which are critical to mitigating ROS damage and promoting oxidative stress tolerance. Similarly, many DEGs acted as *trans*-targets of DELs. Notably, stress related genes involved in protein serine/threonine kinase activity, abscisic acid signalling pathways, lignification and inositol metabolism were identified.

The consistent alignment of gene functions with known stress mitigation pathways supports the biological relevance of the results. Given the exploratory nature of this study, all conclusions drawn (particularly regarding specific lncRNA-gene interactions) should be tested in future experiments. This study lays a foundation for future investigations into lncRNA-mediated regulation of stress responses in *Datura metel*, while providing additional evidence for the potential role of lncRNAs in chemical priming of plants to acquire abiotic stress tolerance.

There is mounting evidence suggesting that the abiotic stresses mentioned throughout this paper will become more prevalent in the coming years as climate change worsens. Understanding the many regulatory mechanisms, including those involving lncRNAs, which control and promote adaptive responses to stress in different plant species is important for finding ways to maintain plant productivity and produce plant species with reduced susceptibility to stress, through genetic or chemical intervention.

**LIST OF ABBREVIATIONS**

ncRNA: non-coding RNA

lncRNA: long non-coding RNA

SA: salicylic acid

DEL: differentially expressed lncRNA

DEG: differentially expressed gene

GO: gene ontology

KO: KEGG orthology

KEGG: Kyoto Encyclopedia of Genes and Genomes

ROS: reactive oxidative species

AtVSP2: *Arabidopsis* vegetative storage protein 2

AP2/ERF: apetala 2/ethylene-responsive factor

ABA: abscisic acid

SnRK2: SNF1-related kinase 2

AREB/ABF: ABRE-binding protein/factor

ABRE: ABA-responsive element

DREB: DRE-binding protein

JA: jasmonic acid

MAPK: mitogen activated protein kinases

PA: phosphatidic acid

SOS1: salt overly sensitive 1

GST: glutathione transferase

TNT: 2,4,6-trinitrotoluene

KAR: Karrikin

KAI2: Karrikin insensitive2

ACO: 1-aminocyclopropane-1-carboxylate oxidase

NO: nitric oxide

ITPK: inositol-1,3,4-trisphosphate 5/6-kinase

AlaAT: alanine aminotransferase

GABA: gamma-aminobutyric acid

POP2: pollen-Pistil Incompatibility 2

## DECLARATIONS

**Ethics approval and consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Availability of data and materials:** The RNA-seq datasets analyzed in this study were publicly available from the NCBI Sequence Read Archive (SRA) under accession numbers SRX17976629 (normal leaf tissue) and SRX17976630 (salicylic acid–treated leaf tissue). The processed data generated in this study, including raw counts, FPKM values, NoiSeq outputs, and the FASTA file of identified lncRNAs, have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE316910 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE316910). These GEO data are

currently private; the reviewer access token is ytetgiqsbderrod. Supplementary data tables supporting the findings of this study are included in Additional File 1, which contains three sheets (Table S1–S3). All other data supporting the conclusions of this study are available from the corresponding author upon reasonable request.

**Competing interests:** The authors declare that they have no competing interests.

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**Authors' contributions:** I.I. carried out the research, gathered data from literature and wrote the manuscript. G.P provided guidance and supervision for Data analysis. M.H. provided guidance, proofread, and supervised the research work. All authors read and approved the final manuscript.

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