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ORIGINAL ARTICLE

# Dynamic water relations and alternative splicing in common bean under progressive soil drought and recovery

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## Abstract

***Key message*** **Drought stress induces widespread alternative splicing in common bean, predominantly through intron retention, uncovering numerous responsive genes and laying the foundation for deciphering molecular networks and breeding for drought resilience.**

**Abstract** Alternative splicing (AS), a post-transcriptional regulatory mechanism, plays a pivotal role in regulating plant stress tolerance by diversifying transcriptomes and the resultant proteomes. Despite its well-documented role in model species, the dynamics of drought-induced AS and their functional relevance in common bean (*Phaseolus vulgaris* L.), a globally vital legume crop, remain largely unexplored. To address this knowledge gap, we employed an integrated approach combining advanced physiological phenotyping, high-resolution PacBio Isoform Sequencing (Iso-seq), and Illumina RNA-Seq to systematically characterize water relations and AS landscapes in common bean leaves under progressive soil drought (light, moderate, severe) and subsequent recovery. Our analysis demonstrated that, across all conditions, intron retention (IR) emerges as the predominant AS type, accounting for ~ 90% of all events, followed by alternative 3' splice sites (A3SS), alternative 5' splice sites (A5SS), and exon skipping (ES). We uncovered 312, 601, and 492 differentially alternative spliced genes (DAGs) responsive to light, moderate, and severe drought stress, respectively. This study not only provides a global landscape of AS regulation in drought-stressed common bean but establishes a foundation for deciphering AS-mediated molecular networks underlying drought adaptation. The integrated dataset serves as a valuable resource for future genomic annotation and precision breeding strategies aimed at enhancing drought resilience in legumes.

## Introduction

Global agriculture is facing dramatic climatic changes including extremely high temperatures and intense drought stress, which often occur simultaneously under the natural environment (Malambane et al. 2023; Ali et al. 2025; Mohan et al. 2025). In the last three decades, vast researches have been invested in improving plant responses to various stresses (Zhang et al. 2022). Nevertheless, the bench-to-field transfer rate (ratio of patents to marketed commercial seeds) of abiotic stress-resistant crops is very low, due to the high complexity of dynamic plant-environment interactions (Kumar et al. 2021). Drought affects several aspects of plant growth and development, including germination, shoot and root development, photosynthesis, and reproducing (Razi & Muneer, 2021; Sato et al. 2024). Due to global climate change, drought has become one of the most uncontrolled and unpredicted factors continuously limiting crop production (Amine-Khodja et al. 2022; Liu et al. 2023).

Under drought stress, post-transcriptional regulatory mechanisms, particularly AS, play crucial roles in modulating gene expression and enhancing plant adaptation (Hussain et al. 2023). AS generates multiple mature mRNA isoforms from a single gene by selecting different splice sites, including intron retention (IR), exon skipping (ES), alternative 5' splice sites (A5SS), and alternative 3' splice sites (A3SS) (Lee et al. 2023; Rodriguez Gallo et al. 2023). Many AS events introduce premature termination codons (PTCs), leading to transcript degradation via nonsense-mediated decay (NMD) or the production of truncated proteins (Gao et al. 2022; Luha et al. 2024). Recent studies indicate that

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AS is widespread in plants, affecting up to 60% of multi-exon genes, and is highly responsive to developmental cues as well as biotic and abiotic stresses (Boulanger et al. 2023; Liu et al. 2024). It has been widely observed that drought stress markedly enhances the occurrence of AS events. In *Arabidopsis*, drought induces AS in numerous genes involved in stress signaling and response pathways (Le Cong Huyen Bao Phan et al. 2022). In rice, AS contributes to drought tolerance by regulating gene expression and diversifying protein function, such as identified two distinct *OsbHLH59* transcripts, namely, *OsbHLH59.1* and *OsbHLH59.2*, with differential expression patterns under drought stress. *OsbHLH59.1* and *OsbHLH59.2* both positively regulate drought tolerance in rice, with *OsbHLH59.2* exhibiting stronger drought resistance (Ning et al. 2025). Notably, the study also found that the alternative splicing of *OsbHLH59* undergoes dynamic changes after rehydration, suggesting that alternative splicing not only responds to drought stress but also participates in the molecular regulation during the process of water recovery, implying that splicing variations may possess environmental responsiveness and functional plasticity (Ning et al. 2025). Collectively, these findings underscore both the conservation and diversification of AS as a core post-transcriptional regulatory mechanism in plant drought responses.

Common bean (*Phaseolus vulgaris* L.) is a globally vital legume crop and importance source of protein and micronutrients for humans, especially in the traditional diets of Latin America and Africa (Hu et al. 2025). Growth and yield of common bean are often limited by multiple abiotic stresses, with drought being one of the most complex and damaging stress factors (Wu et al. 2024; Buckley et al. 2025). About 60% of the world's common bean is located on drought-prone agricultural land and lack irrigation systems, and unexpected drought periods could reduce production by up to 80% (Martínez-Barradas et al. 2024). Although previous studies have revealed the role of AS in plants drought stress response, research on legume crops remains limited. For example, Syed et al. (2015) utilized RNA-seq technology to discover in soybean that drought stress can induce tissue-specific alternative splicing in thousands of genes, which are enriched in key pathways such as ABA signaling and transcriptional regulation. Iñiguez et al. (2017) conducted a study to analyze 157 publicly available RNA-seq libraries of common bean, aiming to identify and characterize AS events, thereby providing useful data for understanding the occurrence patterns of AS in common bean. In this study, we aim to systematically identify AS events in common bean under drought stress using both SMRT and Illumina RNA-seq technologies, combined with physiological data to elucidate the regulatory mechanisms of AS in response to drought. By analyzing dynamic AS changes across different stress levels (light, moderate, and severe) and recovery stages, this work not only offers a valuable data resource for genome annotation in common bean but also provides a theoretical foundation for enhancing drought tolerance in this crop.

## Material and methods

### Plant materials & growth conditions

The commercial common bean cultivar 'ZheYun No.3', used for physiological phenotyping and alternative splicing analysis in this study, was provided by the Zhejiang Academy of Agricultural Sciences (Hangzhou, China). Physiological phenotyping experiments employed the PlantArray platform, a high-throughput phenotyping system featuring automated feedback irrigation to precisely control water conditions for individual plants (Sun et al. 2024, 2025). Plants were grown under natural atmospheric vapor pressure deficit (VPD) and radiation, with temperatures maintained between 20℃ and 36℃. This system enables continuous monitoring of functional growth status and parameters under diverse stress and soil conditions (Fang et al. 2023). The five sampling stages were defined based on real-time VWC values continuously monitored by the PlantArray system to ensure objective and reproducible classification of drought severity. Specifically, the stages were defined as follows: well-watered (WW, VWC 0.25–0.32), light drought (LD, 0.20–0.25), moderate drought (MD, 0.15–0.20), severe drought (SD, 0.10–0.15), and recovery (RC, VWC restored to the WW range after rewatering). Leaf samples were collected at each stress phase, corresponding to the time points indicated in Fig. 1 (A02/WW: 10/29; B14/LD: 11/01; C09/MD: 11/04; D08/SD: 11/07; D12/RC: 11/08). The fully expanded trifoliate leaves (from the top) were collected from each sampled plant to ensure developmental uniformity. Leaf tissues from three individual plants grown under identical conditions in the PlantArray system were pooled to minimize individual variation and to obtain sufficient RNA for sequencing.

### RNA extraction and quantification

Total RNA was isolated using the RNAprep Pure Plant Kit (TIANGEN, Cat. No. DP441) following the manufacturer's protocol. RNA purity and concentration were initially assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA) based on the OD260/280 ratio. Subsequently, RNA integrity was evaluated with an RNA Nano 6000 Assay Kit on an Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA), and RNA degradation/contamination was checked on 1% agarose gels. Construct a cDNA library for subsequent SMRT or Illumina sequencing.

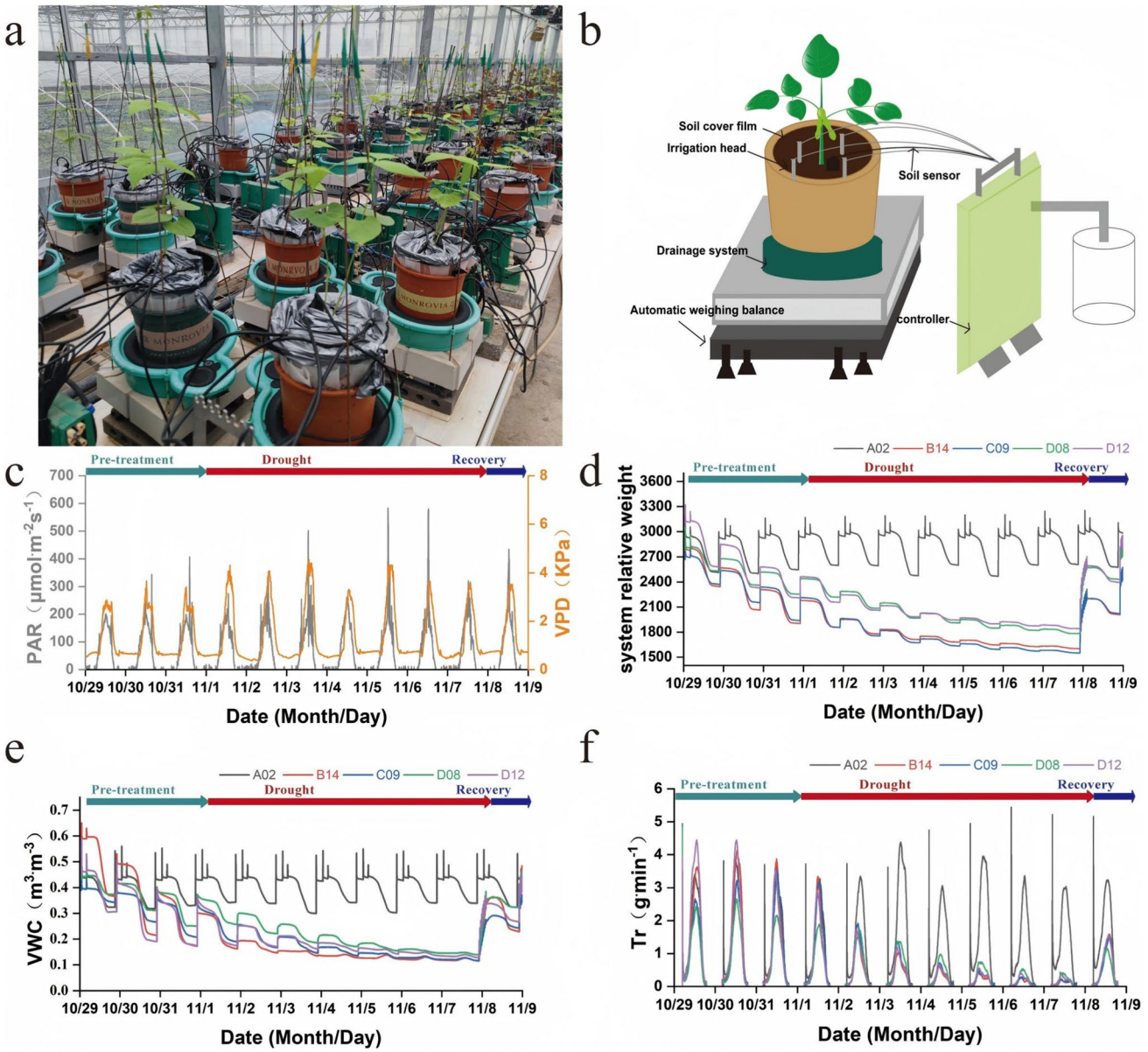



**Fig. 1** Dynamics of common bean water relations under progressive drought and rehydration. **a** and **b** The lysimetric system where randomized experimental array consisting of multiple measuring units loaded with common bean were set up. The system is designed to monitor and analyze various physiological parameters of plants under controlled conditions. The experimental units are arranged in a specific configuration to facilitate precise measurements and data collection. **c** The environment parameters of common bean as recorded by the Plantarray physiological phenotyping system. VPD and photosynthetically active radiation (PAR) conditions during the course of the experiment. **d** Dynamics of system weight during the course of the experiment, which consisted of the pre-treatment, drought stress, and recovery phases. **e** dynamics of soil volumetric water content (VWC) during the test period. **f** Dynamics of transpiration rates (Tr) of common bean. Transpiration rate fluctuates due to irrigation, light intensity, air humidity and other factors. The codes (A02, B14, C09, D08, D12) are specific pot/sample identifiers within the PlantArray system corresponding to the plants sampled for a given treatment at a specific time point. These data help to understand how common bean respond to environmental changes and their water use efficiency

## PacBio (Iso-Seq) library preparation and sequencing

In the process of SMRT library construction, mRNA was purified from the total RNA mixture of common bean. Full-length cDNA was then synthesized using the SMARTer PCR cDNA Synthesis Kit according to the PacBio Isoform Sequencing (Iso-Seq) protocol. The resulting cDNA was amplified by PCR, and the remaining overhangs were converted to blunt ends using exonuclease/polymerase. The DNA fragments were adenylated at their 3'ends, and NEBNext Adaptors featuring a hairpin loop

structure were ligated. Finally, SMRT sequencing was performed on a Pacific Biosciences Sequel II system.

### Illumina RNA-seq library preparation and sequencing

RNA was isolated from leaf samples under treatment conditions using the aforementioned protocol, with three replicates per treatment (Jiang et al. 2024; Ke et al. 2025). Libraries were constructed starting with 1 μg of total RNA. mRNA was isolated via poly(A) selection using magnetic beads and subsequently fragmented enzymatically using the TruSeq™ RNA Sample Prep Kit. Double-stranded cDNA synthesis was performed, followed by end repair, adenylation of 3'ends, and ligation of indexed adapters (TruSeq™ RNA Sample Prep Kit). Libraries were enriched by PCR amplification for 15 cycles. Target fragments were size-selected by excising the appropriate band from the 2% agarose gel. The library was quantified using the TBS380 fluorescence quantitative instrument in conjunction with the Picogreen reagent, and clone clusters were constructed through bridge amplification. Ultimately, paired-end sequencing (2 × 150 bp) was performed on the Illumina NovaSeq 6000 platform.

### Raw data analysis and quality control of Iso-seq

The Iso-seq pipeline developed by Pacific Biosciences (http://www.pacb.com) was used for the analysis of raw reads from sequencing. The joined subreads were disconnected, and joint sequences that were < 50 bp were removed, resulting in clean data. The obtained clean reads were processed into error-corrected circular consensus sequences (CCS) using a stringent standard (Full passes ≥ 2, Predicted accuracy ≥ 0.8). Then, the full-length, non-chimeric (FLNC) transcripts were determined by searching for poly-A tail signals and the 5' and 3' cDNA primers in CCSs. Iterative clustering for error correction (ICE) was used to obtain consensus isoforms, and FL consensus sequences from ICE were polished using Quiver. High-quality FL transcripts were classified as those with a post-correction accuracy criterion surpassing 99% and mapped to the common bean ZH13 reference genome (https://phytozome-next.jgi.doe.gov/info/Pvulgaris_v2_1) using GMAP software.

### AS detection and stress-responsive AS events identification

The SpliceGrapher software was used to classify the total AS events from Iso-seq datasets. Four main AS event types (IR, A3SS, A5SS, and ES) were extracted from the output file. In addition, the rMATS 4.1.1 software was used to analyze the differential AS events between paired samples from the RNA-seq dataset with the junction count-only algorithm. The differential AS events were defined as those with a false discovery rate (FDR) < 0.05. To comprehensively assess within-sample variations, we established multiple comparison groups, including LD versus WW, MD versus WW, SD versus WW, MD versus LD, SD versus LD, SD versus MD, and RC compared with each drought stage.

### Gene ontology and pathway enrichment analysis

Functional-enrichment analysis including GO and KEGG were performed to identify which DAGs were significantly enriched in GO terms and metabolic pathways at Bonferroni-corrected *P* value ≤ 0.05 compared with the whole-transcriptome background. GO functional enrichment and KEGG pathway analysis were carried out by Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/kobas3).

### RT-PCR Validation of splice variants

Total RNA from common bean root samples under progressive soil drought treatment was extracted as described above. The housekeeping gene *PvUBI* (*Phvul.007G052600*) was employed as the reference for normalization in the delta–delta cycle threshold method, quantifying relative expression levels. For RT-PCR validation, the PCR reactions were performed using 2 × EasyTaq PCR SuperMix (TransGen Biotech, China) with 5 × diluted cDNA as the template. The PCR products were resoluted and visualized in 1.0% agarose gel. Primer pairs used for RT-qPCR and RT-PCR validation are listed in Excel S4.

## Results

### Dynamics of plant water relations under progressive drought and rehydration

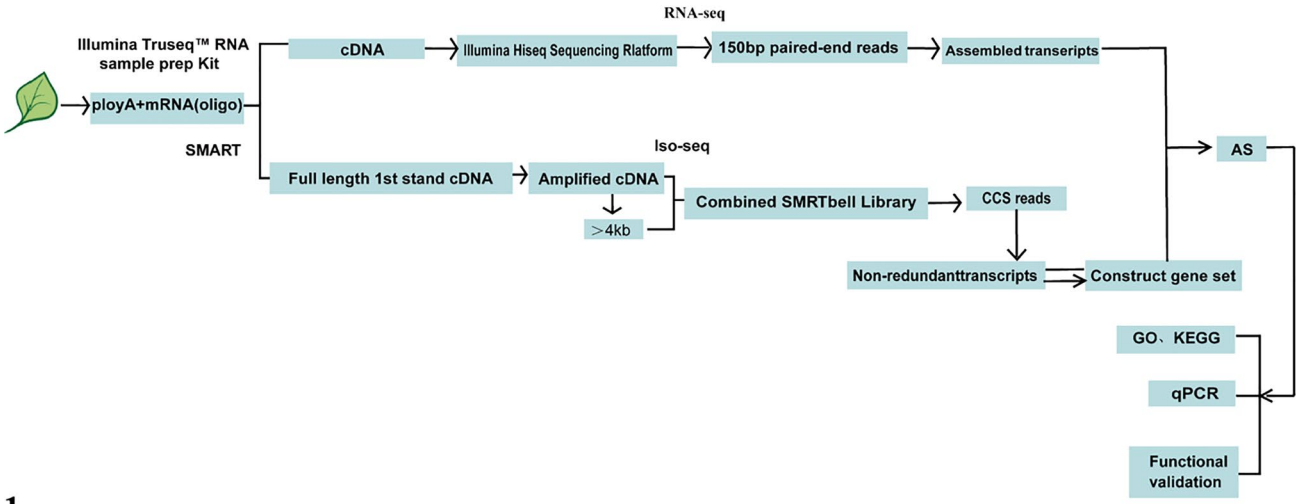
The PlantArray phenotyping platform was employed for continuous monitoring of dynamic water relation phenotypes in common bean plants (Fig. 1a, b). Throughout the experimental period, both photosynthetically active radiation (PAR) and vapor pressure deficit (VPD) exhibited diurnal patterns. PAR reached its peak during the morning hours, whereas VPD peaked in the afternoon (Fig. 1c). Under well-watered conditions, the plants maintained stable system weight and soil volumetric water content (VWC), which fluctuated within normal ranges over the course of the experiment (Fig. 1d, e). In contrast, the drought-stressed plants exhibited a progressive decline in both system weight and VWC following the initiation of drought stress. After rehydration, soil VWC

and system weight recovered rapidly to levels comparable to those of the well-watered controls. Diurnal patterns in plant transpiration rate (Tr) were clearly observed, with Tr typically peaking around midday (Fig. 1f). Notably, although the system weight, VWC, and Tr of drought-treated plants displayed similar dynamic trends, Tr responsed to rehydration differed from the recovery patterns of system relation weight and VWC. While system weight and VWC rapidly returned to pre-treatment levels after rewatering, Tr remained lower than the rate observed prior to drought treatment, though a significant increase occurred. This suggests that plants undergo a phase of physiological adjustment following water restoration.

## Data quality of SMRT- and Illumina-based RNA sequencing

To investigate changes in gene expression and AS during the drought stress and recovery, we employed SMRT (PacBio- Iso-seq) and Illumina (Novaseq 6000) based RNA sequencing for the aforementioned plants grown in PlantArray and subject to different treatment scenarios (WW, LD, MD, SD, and RC) (Fig. 2a). In total, 27.39–34.97 GB of raw data were generated from Iso-seq (Fig. 2b). A total of 254,561–452,693 circular consistent sequence (CCS) reads were obtained, consisting of 232,949–441,740 full-length non-chimeric (FLNC) reads from the Iso-seq datasets. The CCS reads were predominantly distributed in the range of 100–12,000 bp, with a mean length of 1,842–2,753 bp (Fig. S1). The FLNC

a




b

| Sample | Subreads base(G) | Subreads number | Average length | CCS | FL | FLNC | Polished reads number | gmap mapped | Mapped rate | Min_length | Max_length |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WW | 34.59 | 16456980 | 2753 | 316777 | 314037 | 274519 | 99547 | 98668 | 99.12% | 50 | 198405 |
| LD | 34.97 | 24718707 | 1842 | 452693 | 448223 | 441740 | 95091 | 94471 | 99.35% | 50 | 180227 |
| MD | 34.24 | 22920556 | 2056 | 435989 | 431870 | 424893 | 110150 | 109411 | 99.33% | 50 | 202482 |
| SD | 27.39 | 15254197 | 2464 | 254561 | 252306 | 232949 | 85995 | 84015 | 97.70% | 50 | 211884 |
| RC | 30.79 | 14966483 | 2744 | 281113 | 278612 | 258099 | 98783 | 97881 | 99.09% | 50 | 201219 |

**Fig. 2** Workflow used in this study. **a** Leaf samples from two-week-old common bean seedlings under progressive soil drought treatment were pooled for Iso-seq on the PacBio Sequel system and RNA-seq on the Illumina Novaseq™ 6000 platform. **b** The statistics of the Iso-seq data

reads of the severe drought sample was the lowest, indicating that the severe drought stress had a more significant impact on leaf gene expression.

In addition, a total of 669.98 million 150 bp paired-end reads were generated from these samples. Low-quality reads were removed, leaving 662.47 million paired-end reads (98.87% of total paired-end reads) for downstream analysis. On average, 95.74% of the filtered sequencing reads mapped to the common bean reference genome (Excel S1). Subsequently, a total of 50,421 transcripts were identified and their expression was quantified as FPKM value. Expression profiling of 50,421 transcripts within different stages identified 27,433 genes, allowing us to determine patterns of expression at both the level of the gene and at the individual transcript isoform level (Excel S2). Principal component analysis (PCA) of gene expression data clearly separated the samples into three distinct groups, capturing the temporal dynamics of the drought response throughout the experiment (Fig. S2).

## Identification of AS events from Illumina, SMRT and combined data

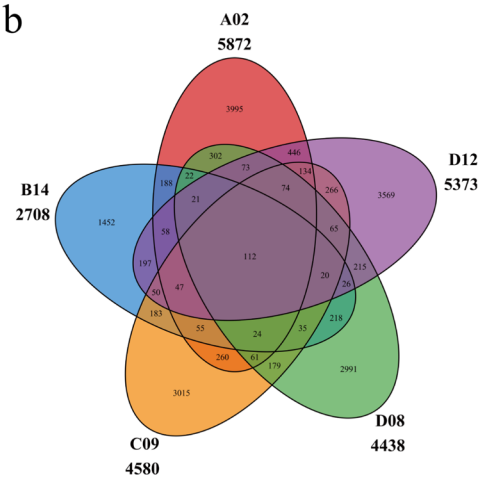
The set of AS events based on full-length transcriptome served as the basis for correction with second-generation sequencing data (Fig. 3a). Our validation analysis revealed a high overall confirmation rate for AS isoforms. Across all samples, a total of 18,353 AS isoforms were identified. After rigorous validation, 13,528 of these were confirmed, yielding a high overall validation rate of 73.71%. Among the individual treatments, the validation rates were consistently high, ranging from 69.40% to 77.14%. Specifically, the MD treatment group exhibited the highest validation rate at 77.14%, while the WW group showed the lowest rate at 69.40%. The other groups (LD, SD, and RC) demonstrated comparable rates of 74.15%, 73.55%, and 73.76%, respectively (Fig. 3b).

From above analysis, a total of 22,505 AS events were identified from 18,353 genes in all samples**.** Among them IR was the most abundant type (20,279, 90%), followed by A3SS (1,187, 5%), A5SS (843, 4%) and ES (196, 1%)

a

| | AS.num | Validated splicing isoform | Validated rate (%) |
|---|---|---|---|
| WW(A02) | 5,872 | 4,075 | 69.40% |
| LD(B14) | 2,708 | 2,008 | 74.15% |
| MD(C09) | 4,580 | 3,533 | 77.14% |
| SD(D08) | 4,438 | 3,264 | 73.55% |
| RC(D12) | 5,373 | 3,963 | 73.76% |
| merge.unique | 18,353 | 13,528 | 73.71% |

b




c

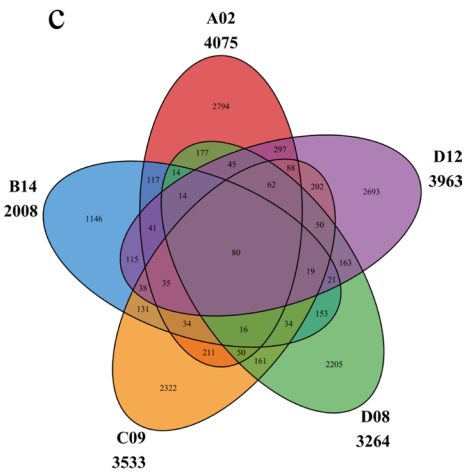



**Fig. 3** Summary of various AS events identified by RNA-Seq and Iso-Seq under progressive drought. **a** Statistical analysis of AS events under five treatments combined with RNA-Seq and Iso-Seq. **b** AS Venn analysis based on three generations of full transcriptome data. c Reliable AS Venn analysis of second-generation transcriptome data after filtering

(Table 1). These results were consistent with those of previous reports in other plant (Liu et al. 2024). During the whole experiments in common bean plants maximum number of AS events were identified in CK (4998, 22.2%) in compare to other conditions MD (4859, 21.6%), RC (4822, 21.4%), SD (4149 18.4%), and LD (3677, 16.3%) (Table 1).

## Identification of stress-responsive AS events in common bean during different treatment

To investigate the dynamics of AS in response to drought stress, we analyzed differentially alternative spliced genes (DAGs) across multiple treatment comparisons. The data revealed significant dynamics in AS regulation under drought stress and during recovery (Fig. 4). For instance, the comparison between LD and WW revealed 361 DAGs, with 138 upregulated and 223 downregulated. In contrast, the MD versus WW comparison showed a higher number of DAGs at 585, with 512 upregulated and 73 downregulated, indicating a more pronounced shift in splicing patterns. The MD versus LD comparison further escalated the number of DAGs to 922, with 658 upregulated and 264 downregulated, suggesting a substantial difference in splicing regulation between these two conditions. Comparisons involving SD (SD vs. WW, SD vs. LD, and SD vs. MD) consistently showed high numbers of DAGs, with SD versus WW having the highest at 2077 DAGs (1131 upregulated and 946 downregulated), indicating a significant impact of SD on AS. The RC comparisons (RC vs. WW, RC vs. LD, RC vs. MD, and RC vs. SD) also demonstrated substantial numbers of DAGs, with RC versus LD showing the highest at 2345 DAGs (1841 upregulated and 504 downregulated), highlighting the extensive splicing changes induced by RC.

By conducting temporal clustering analysis on DAGs in the common bean under five treatment conditions, we categorized the genes into 20 clusters, each exhibiting distinct dynamic expression patterns (Fig. S3). Collectively, as drought severity increased, the expression levels of most genes displayed a gradual upward trend. Following rewatering, partial recovery of expression was observed in a subset of genes. Several clusters (such as Cluster 6, Cluster 10, Cluster 11, Cluster 15, and Cluster 18) demonstrated pronounced stage-specific expression behaviors. For instance, certain clusters (such as Cluster 1, Cluster 3, Cluster 8, Cluster 15, and Cluster 17) underwent significant changes under light drought and continued to be either upregulated or downregulated thereafter. Other clusters (such as Cluster 4, Cluster 5, Cluster 9, Cluster 16, and Cluster 18) exhibited notable expression alterations only under moderate or severe drought conditions. Additionally, some clusters (e.g., Cluster 7 and Cluster 11) rapidly returned to near-baseline expression levels after rewatering, suggesting their potential involvement in post-drought recovery processes. These varied expression dynamics across clusters reflect multi-layered transcriptional regulatory strategies employed by the common bean in response to drought stress and subsequent recovery.

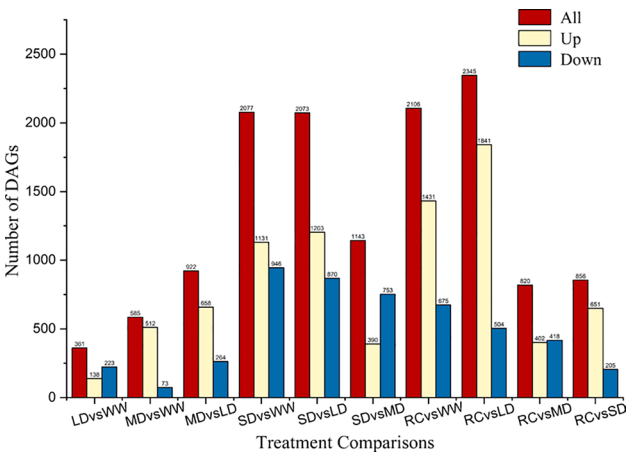



**Fig. 4** Distribution of DAGs among progressive drought and comparisons

Next, we analyzed unique AS under different treatments (Excel S3). WW remained the most prolific, producing 2794 total special AS events and 683 unique transcripts indicating

**Table 1** Statistics of different AS event types of RNA-Seq and Iso-Seq datasets

| Type | Structure | WW | LD | MD | SD | RC | Total |
|---|---|---|---|---|---|---|---|
| IR | | 4571 | 3255 | 4375 | 3772 | 4306 | 20,279 |
| A3SS | | 231 | 208 | 262 | 200 | 286 | 1,187 |
| A5SS | | 158 | 174 | 178 | 143 | 190 | 843 |
| ES | | 38 | 40 | 44 | 34 | 40 | 196 |
| | Total | 4998 | 3677 | 4859 | 4149 | 4822 | 22,505 |

a rich, balanced program responsive to both housekeeping and specialized cues. RC trails closely with 2693 total special AS events and 632 unique events, implying that while splice-site choices were conserved. LD exhibited the sharpest contraction: total events falled to 1,146, unique AS genes dropped to 312. SD and MD occupied intermediate ground: SD recorded 2205 total special events, 492 unique, and MD yielded 2322 total, 601 unique. The occurrence of unique AS events response to drought signals—there were 173 in LD, 278 in SD, and 368 in MD. Interestingly, RC was relatively close to WW, with 367 and 420, respectively (Fig. 5a, b). Overall, the data revealed the loss and gain of splicing variants during the progressive drought and rewatering process of common bean: LD produced the fewest splicing events, SD/MD introduced more splicing events, RC had more specific events, but had not yet fully restored the diversity of WW.

To explore the correlation between physiological indicators and the expression of key DAGs, we analyzed the expression distribution of key DAS genes across the drought and recovery stages (Excel S5). The median of the violin plot exhibits a positive association with the Tr during the water stress stages (LD, MD, SD), with median gene expression declining as Tr decreased. Consistently, both Tr and median gene expression reached their lowest levels at the SD stage. During the rehydration stage (RC), Tr rapidly rebounded to 1.336, indicating rapid recovery and enhancement of transpiration capacity after rewatering (Fig. S4, Excel S6). In contrast, median gene expression did not increase concurrently, suggesting a lag in transcriptional recovery relative to physiological restoration. This delay may reflect gradual post-transcriptional adjustment mediated by alternative splicing following rehydration.

## Functional analysis of drought-responsive AS events in common bean

To investigate the biological functions of DAGs under drought stress, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed. Based on the leaf DAGs in our RNA-seq dataset, as shown in Fig. 6a, b, the patterns of enriched GO terms among DAGs under the five treatments (WW, LD, MD, SD, RC) are similar. For example, in the biological process (BP) category, terms such as "single-organism process"(GO: 0044763) and "cellular metabolic process" (GO: 0009987) are enriched; in the molecular function (MF) category, terms including "catalytic activity" (GO: 0003824), "binding" (GO: 0005488), and "transporter activity" (GO: 0005215) are enriched; and in the cellular component (CC) category, terms like "cell part" (GO: 0044464) and "organelle" (GO: 0006996) are enriched. These are the common GO terms with relatively large numbers of DAGs (Fig. 6a).

Meanwhile, we performed KEGG enrichment analysis and found that the KEGG pathway enriched by the largest number of DAGs in WW was "biosynthesis of factors" (KO: 01240); in LD, it was "carbon metabolism"(KO: 01200); in MD, it was "metabolic pathways" (KO: 01100); in SD, it was "ubiquitin-mediated proteolysis" (KO: 04120); and in RC, it was "biosynthesis of factors" (KO: 01240). Our research results demonstrated that these genes respond to drought stress in common bean and undergo distinct splicing patterns (Fig. 6b).

## PCR validation of AS events

IR was identified as the predominant AS event in both our RNA-seq and Iso-seq datasets. To experimentally validate the prevalence and functional implications of IR, we selected four drought-associated genes (*Phvul.009G167100*, *Phvul.011G035200*, *Phvul.003G191900*, and *Phvul.009G163400*), which collectively harbor 15 splice variants. These four DAGs were exclusively identified as being differentially spliced under stringent drought conditions. We subsequently conducted RT-PCR experiments to validate the predicted IR-type AS events in these four DAGs. The AS events in the selected DAGs are predicted to introduce premature termination codons, potentially leading to alterations in the coding sequence (CDS) length and thus impacting protein function. The RT-PCR and sequencing verification (*Phvul.003G191900*, *Phvul.009G163400*) results confirmed that the amplified fragment sizes for each splice variant corresponded precisely to the predicted

a

| Treatment | Total Special Events | Unique AS |
|---|---|---|
| Well-Watered (WW) | 2794 | 683 |
| Light drought (LD) | 1146 | 312 |
| Moderate drought (MD) | 2322 | 601 |
| Severe drought (SD) | 2205 | 492 |
| Recovery (RC) | 2693 | 632 |

b

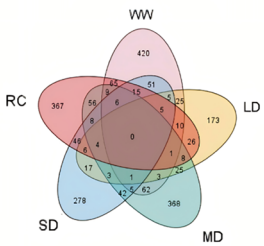



**Fig. 5** Numbers of different AS events in DAGs. **a** Unique AS events characteristics of common bean under progressive drought treatment. **b** Venn analysis of unique AS events under progressive drought treatment

sequences, thereby verifying the accuracy of the alternative splicing events in these drought-specific genes and providing a reliable molecular basis for the functional study of them in drought response (Fig. 7, Fig. S5).

## Discussion

### AS as a dynamic regulatory hub in drought adaptation of common bean

Our study establishes alternative splicing (AS) as a central post-transcriptional mechanism shaping common bean responses to progressive drought. The dominance of intron retention (IR) across all drought stages aligns with reports in Arabidopsis, maize, and rice, suggesting an evolutionarily conserved strategy to rapidly reprogram gene expression under water deficit (Li & Howell, 2021). IR serves dual functions: both by generating truncated protein isoforms that act as dominant-negative regulators, and by triggering nonsense-mediated decay (NMD) to attenuate stress-responsive transcripts, thereby fine-tuning resource allocation. For instance, IR in NAC and WRKY transcription factors—key drought signaling hubs—likely modulates downstream stress networks, as observed in Arabidopsis DREB2A splice variants (Theisen et al. 2024).

Notably, the progressive increase in DAGs from light (361) to severe drought (2077 DAGs in SD vs. WW) implies a "splicing stress memory" phenomenon, where early AS events may prime molecular machinery for subsequent stress escalation (Fig. 4). This is further evidenced by the persistence of drought-induced isoforms during recovery (RC), suggesting a hysteretic effect that could accelerate reactivation of stress pathways upon recurring drought. Such splicing-mediated memory has been reported in rice osmoprotectant genes and warrants validation under cyclic drought regimes.

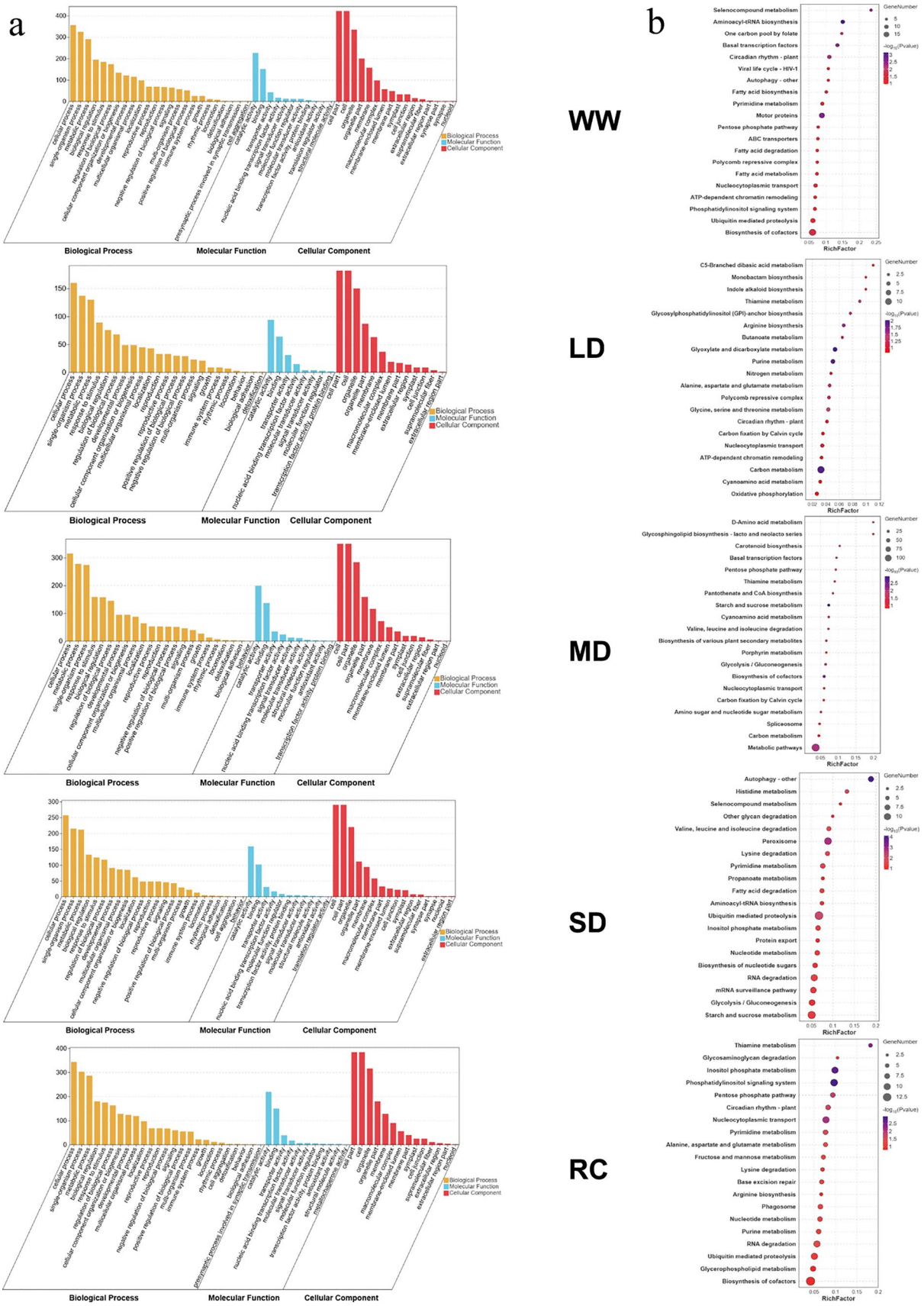
### Stage-specific AS signatures and functional implications

Unique AS events exhibited stage-specific enrichment patterns. Light drought (LD) displayed the fewest unique events (312), reflecting a resource-conserving "thrifty" strategy, whereas moderate (MD) and severe drought (SD) introduced markedly more variants (601 and 492, respectively). Recovery (RC) partially restored splicing diversity (632 unique events) but failed to fully recapitulate the well-watered (WW) state (683 unique events), indicating incomplete homeostatic reset. GO and KEGG analyses revealed that WW-enriched AS genes were associated with housekeeping processes (e.g., "biosynthesis of cofactors"), whereas drought stages prioritized metabolic reprogramming (e.g., "carbon metabolism" in LD, "ubiquitin-mediated proteolysis" in SD). This shift underscores AS-mediated reallocation of cellular resources from growth to stress defense.

Previous studies have showed that core members of the ABA signaling pathway, including enzymes responsible for ABA synthesis (ZEPs), transporters (ABCG), and signal modulators (PP2C), which undergo alternative splicing under drought conditions (Lin et al. 2025). In this study, KEGG and GO enrichment analyses of hormone-related pathways revealed significant enrichment of ABA and auxin pathways among the DAGs. For instance, *Phvul.002G280000* exhibits distinct alternative splicing patterns under moderate and severe drought in common bean. This gene belongs to the *amidase* family and is involved in coordinating the trade-off between plant growth and stress response, by maintaining the homeostatic balance between auxin and ABA (Fenech et al. 2025). Such regulation enables a single gene to produce multiple slightly different mRNA and protein variants, allowing fine-tuned and dynamic adjustments in ABA synthesis, transport, and signaling intensity to adapt to varying degrees of drought (Ma et al. 2025). Nevertheless, the dynamic patterns, molecular mechanisms, and physiological functions of AS in the ABA signaling pathway remain to be fully elucidated. Our catalog of drought-responsive AS events provides novel targets for precision breeding. Unlike traditional approaches focusing on gene knockouts or overexpression, modulating splicing factors could generate "splicing-optimized" cultivars with balanced stress tolerance and yield (Lin et al. 2022). CRISPR-mediated editing of splice sites or regulatory elements in lncRNAs offers a promising strategy. However, pleiotropy remains a concern, as splicing factors often regulate thousands of transcripts. Tissue-specific promoters or synthetic splicing regulators may mitigate off-target effects (Singha et al. 2021; Chennakesavulu et al. 2022; Wilson et al. 2022). Furthermore, exploring natural AS variation across common bean landraces may uncover alleles associated with drought resilience, enabling marker-assisted selection.

### Technical advances and limitations

The integration of Iso-Seq and RNA-Seq overcame individual platform limitations: Iso-Seq resolved full-length isoforms and rare splice junctions, while RNA-Seq provided quantitative depth. However, SMRT's lower throughput may underrepresent low-abundance stress-responsive isoforms, and future studies could leverage Oxford Nanopore's adaptive sampling to enrich drought-specific loci. Critically, transcript-level evidence requires proteomic validation (e.g., ribosome profiling or mass spectrometry) to confirm translational relevance, as only ~ 60% of stress-induced AS transcripts are actively translated in maize.


a
b
WW
LD
MD
SD
RC
Biological Process
Molecular Function
Cellular Component
RichFactor
GeneNumber
-log10(Pvalue)
Selenocompound metabolism
Aminoacyl-tRNA biosynthesis
One carbon pool by folate
Basal transcription factors
Circadian rhythm - plant
Viral life cycle - HIV-1
Autophagy - other
Fatty acid biosynthesis
Pyrimidine metabolism
Motor proteins
Pentose phosphate pathway
ABC transporters
Fatty acid degradation
Polycomb repressive complex
Fatty acid metabolism
Nucleocytoplasmic transport
ATP-dependent chromatin remodeling
Phosphatidylinositol signaling system
Ubiquitin mediated proteolysis
Biosynthesis of cofactors
C5-Branched dibasic acid metabolism
Monobactam biosynthesis
Indole alkaloid biosynthesis
Thiamine metabolism
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis
Arginine biosynthesis
Butanoate metabolism
Glyoxylate and dicarboxylate metabolism
Purine metabolism
Nitrogen metabolism
Alanine, aspartate and glutamate metabolism
Polycomb repressive complex
Glycine, serine and threonine metabolism
Circadian rhythm - plant
Carbon fixation by Calvin cycle
Nucleocytoplasmic transport
ATP-dependent chromatin remodeling
Carbon metabolism
Cyanoamino acid metabolism
Oxidative phosphorylation
D-Amino acid metabolism
Glycosphingolipid biosynthesis - lacto and neolacto series
Carotenoid biosynthesis
Basal transcription factors
Pentose phosphate pathway
Thiamine metabolism
Pantothenate and CoA biosynthesis
Starch and sucrose metabolism
Cyanoamino acid metabolism
Valine, leucine and isoleucine degradation
Biosynthesis of various plant secondary metabolites
Porphyrin metabolism
Glycolysis / Gluconeogenesis
Biosynthesis of cofactors
Nucleocytoplasmic transport
Carbon fixation by Calvin cycle
Amino sugar and nucleotide sugar metabolism
Spliceosome
Carbon metabolism
Metabolic pathways
Autophagy - other
Histidine metabolism
Selenocompound metabolism
Other glycan degradation
Valine, leucine and isoleucine degradation
Peroxisome
Lysine degradation
Pyrimidine metabolism
Propanoate metabolism
Fatty acid degradation
Aminoacyl-tRNA biosynthesis
Ubiquitin mediated proteolysis
Inositol phosphate metabolism
Protein export
Nucleotide metabolism
Biosynthesis of nucleotide sugars
RNA degradation
mRNA surveillance pathway
Glycolysis / Gluconeogenesis
Starch and sucrose metabolism
Thiamine metabolism
Glycosaminoglycan degradation
Inositol phosphate metabolism
Phosphatidylinositol signaling system
Pentose phosphate pathway
Circadian rhythm - plant
Nucleocytoplasmic transport
Pyrimidine metabolism
Alanine, aspartate and glutamate metabolism
Fructose and mannose metabolism
Lysine degradation
Base excision repair
Arginine biosynthesis
Phagosome
Nucleotide metabolism
Purine metabolism
RNA degradation
Ubiquitin mediated proteolysis
Glycerophospholipid metabolism
Biosynthesis of cofactors

◂**Fig. 6** Analyses of GO and KEGG pathways for the stage-specific (unique) DAGs enriched from common bean leaf under progressive drought stress. **a** GO terms associated with unique AS genes enriched under progressive drought stress. **b** KEGG pathways associated with unique AS genes enriched under progressive drought stress

The catalog of drought-responsive AS events provides novel targets for precision breeding (Rosenkranz et al. 2022). Unlike traditional approaches focusing on gene knockouts or overexpression, modulating splicing factors could generate "splicing-optimized" cultivars with balanced stress tolerance and yield (Jin et al. 2020; Lin et al. 2022). CRISPR-mediated editing of splice sites or regulatory elements in lncRNAs offers a promising strategy (Anwar & Kim, 2020). However, pleiotropy remains a concern, as splicing factors often regulate thousands of transcripts. Tissue-specific promoters or synthetic splicing regulators may mitigate off-target effects (Zafar et al. 2020; Yu et al. 2023; Thangaraj et al. 2025). Furthermore, exploring natural variation in AS patterns across common bean landraces could uncover allelic variants linked to drought adaptation, facilitating marker-assisted selection.

## Conclusions

This study provides a comprehensive analysis of alternative splicing (AS) dynamics and its role in drought stress response in common bean. Through an integrative approach combining SMRT and Illumina RNA sequencing, we identified a large number of AS events and splice isoforms differentially expressed under progressive drought conditions and during recovery. By integrating physiological data, we elucidated the regulatory mechanisms of AS in response to drought. The analysis of AS dynamics across different drought severities (light, moderate, severe) and recovery stages not only enriched the genomic annotation of common bean but also provided a theoretical foundation for enhancing their drought tolerance. These findings offer valuable insights for future research and practical applications aimed at improving crop resilience to water-limited conditions.

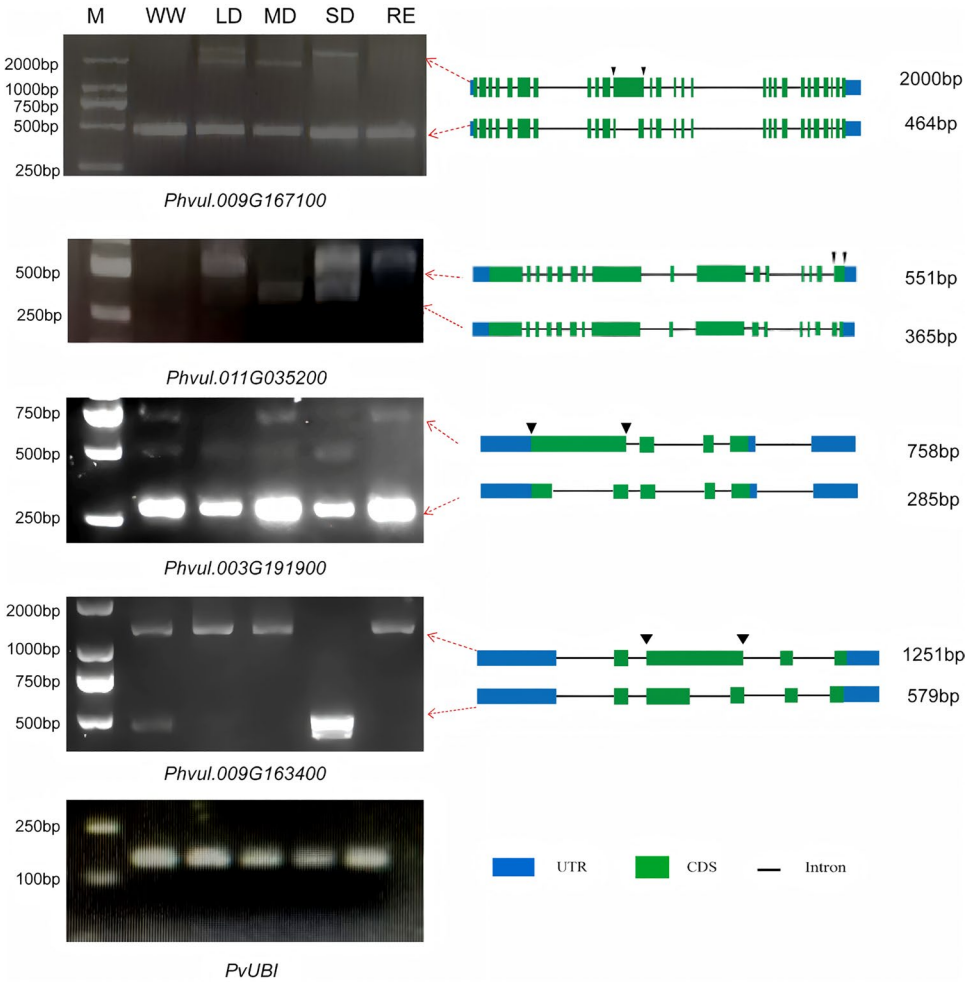



**Fig. 7** PCR validation of AS events of selected DAGs from the RNA-seq dataset. The AS patterns of four DAGs were confirmed by RT-PCR. Exons are denoted by green boxes, untranslated regions by blue boxes, and introns by lines. The black arrowhead indicates the position of primers used for RT-PCR. *PvUBI* was used as an internal control

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**Data availability** The authors declare that all data can be found in this manuscript or in supplementary files and seeds of materials are available upon request.

**Code availability** The authors declare that software application or custom code support their published claims and comply with feld standards.

## Declarations

**Conflict of interest** The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

**Human and animal rights** This study does not include human or animal subjects.

## References

Ali S, Mir RA, Haque MA et al (2025) Exploring physiological and molecular dynamics of drought stress responses in plants: challenges and future directions. Front Plant Sci 16:1565635

Amine-Khodja IR, Boscari A, Riah N et al (2022) Impact of two strains of *Rhizobium leguminosarum* on the adaptation to terminal water deficit of two cultivars *Vicia faba*. Plants (Basel) 11(4):515

Anwar A, Kim JK (2020) Transgenic breeding approaches for improving abiotic stress tolerance: recent progress and future perspectives. Int J Mol Sci 21(8):2695

Boulanger HG, Guo W, Monteiro LFR et al (2023) Co-expression network of heat-response transcripts: A glimpse into how splicing factors impact rice basal thermotolerance. Front Mol Biosci 10:1122201

Buckley TN, Magney TS, Berny Mier Y et al (2025) Diversity in stomatal and hydraulic responses to post-flowering drought in common (*Phaseolus vulgaris*) and tepary (*P. acutifolius*) beans. Plant Cell Environ 48(1):51–64

Chennakesavulu K, Singh H, Trivedi PK et al (2022) State-of-the-art in CRISPR technology and engineering drought, salinity, and thermo-tolerant crop plants. Plant Cell Rep 41(3):815–831

Fang P, Li M, Guo Q et al (2023) Genome-wide analysis of the SMXL gene family in common bean and identification of karrikin-responsive *PvSMXL2* as a negative regulator of PEG-induced drought stress. Gene 887:147741

Fenech M, Brumos J, Pěnčík A et al (2025) The CYP71A, NIT, AMI, and IAMH gene families are dispensable for indole-3-acetaldoxime-mediated auxin biosynthesis in Arabidopsis. Plant Cell 37(11):koaf242

Gao Y, Pang AP, Ma L et al (2022) Intron retention coupled with nonsense-mediated decay is involved in cellulase biosynthesis in cellulolytic fungi. Biotechnol Biofuels Bioprod 15(1):53

Hu H, Yuan X, Saini DK et al (2025) A panomics-driven framework for the improvement of major food legume crops: advances, challenges, and future prospects. Hortic Res 12(7):uhaf091

Hussain SS, Abbas M, Abbas S et al (2023) Alternative splicing: transcriptional regulatory network in agroforestry. Front Plant Sci 14:1158965

Iñiguez LP, Ramírez M, Barbazuk WB et al (2017) Identification and analysis of alternative splicing events in *Phaseolus vulgaris* and *Glycine max*. BMC Genomics 18(1):1–17

Jiang R, Sun T, Shi Z et al (2024) Leveraging "golden-hour" WUE for developing superior vegetable varieties with optimal water-saving and growth traits. Veg Res 4:e002

Jin H, Lin J, Zhu Z (2020) Pif4 and hookless1 impinge on common transcriptome and isoform regulation in thermomorphogenesis. Plant Commun 1(2):100034

Ke XB, Yao J, Jiang ZH et al (2025) Recover and surpass: the mechanisms of plants transition upon rehydration from drought. Plant Stress 15:100782

Kumar V, Thakur JK, Prasad M (2021) Histone acetylation dynamics regulating plant development and stress responses. Cell Mol Life Sci 78(10):4467–4486

Lee KC, Kim YC, Kim JK et al (2023) Regulation of flowering time and other developmental plasticities by 3' splicing factor-mediated alternative splicing in **Arabidopsis thaliana**. Plants 12(19):3508

Li Z, Howell SH (2021) Heat stress responses and thermotolerance in maize. Int J Mol Sci 22(2):948

Lin J, Shi J, Zhang Z et al (2022) Plant AFC2 kinase desensitizes thermomorphogenesis through modulation of alternative splicing. iScience 25(4):104051

Lin F, Cai Y, Yang S et al (2025) Alternative Splicing Events and ABA Hormone Regulation in Drought Response of *Hippophae gyantsensis* L. Genes (Basel) 16(3):350

Lippmann R, Babben S, Menger A et al (2019) Development of wild and cultivated plants under global warming conditions. Curr Biol 29(24):R1326–R1338

Liu S, Zenda T, Tian Z et al (2023) Metabolic pathways engineering for drought or/and heat tolerance in cereals. Front Plant Sci 14:1111875

Liu X, Li M, Chen T et al (2024) A global survey of bicarbonate stress-induced pre-mRNA alternative splicing in soybean via integrative analysis of Iso-seq and RNA-seq. Int J Biol Macromol 278(4):135067

Luha R, Rana V, Vainstein A et al (2024) Nonsense-mediated mRNA decay pathway in plants under stress: general gene regulatory mechanism and advances. Planta 259(3):51

Ma S, Li D, Yang Z et al (2025) Regulation of alternative splicing in the ABA signaling pathway of plants. New Phytol. https://doi.org/10.1111/nph.70846

Malambane G, Madumane K, Sewelo LT et al (2023) Drought stress tolerance mechanisms and their potential common indicators to salinity, insights from the wild watermelon (*Citrullus lanatus*): a review. Front Plant Sci 13:1074395

Martínez-Barradas V, Galbiati M, Barco-Rubio F et al (2024) *PvMYB60* gene, a candidate for drought tolerance improvement in common bean in a climate change context. Biol Res 57(1):52

Mohan VR, MacDonald MT, Abbey L (2025) Impact of water deficit stress on *brassica* crops: growth and yield, physiological and biochemical responses. Plants (Basel) 14(13):1942

Ning M, Li Q, Wang Y et al (2025) Alternative splicing drives the functional diversification of a bHLH transcription factor in the control of growth and drought tolerance in rice. Sci Bull 70(2):153–156

Phan TLCHB, Crepin N, Rolland F et al (2022) Two trehalase isoforms, produced from a single transcript, regulate drought stress tolerance in *Arabidopsis thaliana*. Plant Mol Biol 108(6):531–547

Razi K, Muneer S (2021) Drought stress-induced physiological mechanisms, signaling pathways and molecular response of chloroplasts in common vegetable crops. Crit Rev Biotechnol 41(5):669–691

Rodriguez Gallo MC, Uhrig RG (2023) Phosphorylation mediated regulation of RNA splicing in plants. Front Plant Sci 14:1249057

Rosenkranz RRE, Ullrich S, Löchli K et al (2022) Relevance and regulation of alternative splicing in plant heat stress response: current understanding and future directions. Front Plant Sci 13:911277

Sato H, Mizoi J, Shinozaki K et al (2024) Complex plant responses to drought and heat stress under climate change. Plant J 117(6):1873–1892

Singha DL, Das D, Sarki YN et al (2021) Harnessing tissue-specific genome editing in plants through CRISPR/Cas system: current state and future prospects. Planta 255(1):28

Sun T, Shi Z, Jiang RJ et al (2024) Converging functional phenotyping with systems mapping to illuminate the genotype-phenotype associations. Horticulture Research 11(12):uhae256

Sun T, Lu C, Shi Z et al (2025) PlantRing: a high-throughput wearable sensor system for decoding plant growth, water relations and innovating irrigation. Plant Commun 6(5):101322

Syed NH, Prince SJ, Mutava RN (2015) Core clock, SUB1, and ABAR genes mediate flooding and drought responses via alternative splicing in soybean. J Exp Bot 66(22):7129–7149

Thangaraj A, Kaul R, Sharda S et al (2025) Revolutionizing cotton cultivation: a comprehensive review of genome editing technologies and their impact on breeding and production. Biochem Biophys Res Commun 742:151084

Theisen FF, Prestel A, Elkjær S et al (2024) Molecular switching in transcription through splicing and proline-isomerization regulates stress responses in plants. Nat Commun 15(1):592

Wilson EA, Mao T, Zhong H (2022) Labeling endogenous proteins using CRISPR-mediated insertion of exon (CRISPIE). Bio Protoc 12(5):e4343

Wu X, Chen S, Zhang Z et al (2024) A viral small interfering RNA-host plant mRNA pathway modulates virus-induced drought tolerance by enhancing autophagy. Plant Cell 36(9):3219–3236

Yu L, Zhang H, Guan R et al (2023) Genome-wide tissue-specific genes identification for novel tissue-specific promoters discovery in soybean. Genes (Basel) 14(6):1150

Zafar SA, Zaidi SS, Gaba Y et al (2020) Engineering abiotic stress tolerance via CRISPR/ Cas-mediated genome editing. J Exp Bot 71(2):470–479

Zhang H, Zhu J, Gong Z et al (2022) Abiotic stress responses in plants. Nat Rev Genet 23(2):104–119