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TRANSGENIC SOYBEAN HARBORING *Isopentenyl transferase 10* (*GmIPT10*) DISPLAYS IMPROVED DROUGHT RESILIENCE

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ABSTRACT

Soybean (*Glycine max* (L.) Merrill) is an important global source of plant-based oil and nutrition yet is highly sensitive to water deficit conditions, which significantly impair its growth, development and productivity. In this study, transgenic soybean lines carrying a soybean cytokinin metabolic gene *Isopentenyl transferase* (*GmIPT10*), which was previously identified as a drought-associated gene, were evaluated for their resilience under drought stress conditions. Following the water withholding treatments at the vegetative and early flowering stages, the transgenic plants exhibited reduced stress indicators compared with the control counterparts, including approximately 27−51% lower malondialdehyde (MDA) levels and 33−55% less cellular ion leakage in the leaf tissues. Under the adverse conditions, RT-qPCR analysis revealed elevated expression levels of several drought-responsive marker genes (*GmRD20A*, *GmERD1*) and antioxidant-related genes (*GmPOD*, *GmCAT*, *GmGST4*) but more suppressed expression in the photosynthesis-negative regulator *GmSGR1* in the transgenic plants. Additionally, the transformants maintained a higher proportion of mature pods containing two or three seeds, indicating enhanced yield potential under stress. Collectively, these findings suggest a better stress tolerance of *IPT*-transgenic soybeans, which is partially achieved by better plant protection from drought-induced damages, especially enhanced defense against oxidative stress, thereby supporting plant functions under water-limited conditions. This research highlights the biotechnological potential of the soybean *GmIPT10* gene in enhancing soybean tolerance and its productivity under water stress conditions.

Keywords: Cytokinin, drought tolerance, osmotic stress, stress indicator, water deficit.

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INTRODUCTION

Among leguminous crops, *Glycine max* (L.) Merrill (soybean) is one of the most important members in terms of economical and nutritional values. In addition to being a major source of vegetative oil, soybean is particularly rich in proteins, vitamins, unsaturated fatty acids and health-promoting isoflavones (Arya et al., 2021). Soybean is cultivated across diverse regions worldwide; however, both its productivity and seed compositions are highly influenced by the environmental constraints such as temperature, water, light intensity, soil pH and salinity (Rogovska et al., 2007; Sobko et al., 2020; Sato et al., 2024). Such external parameters, particularly drought, are currently more challenging to manage and predict as a consequence of climate change. Water scarcity is a critical abiotic stress factor that negatively affects soybean throughout its entire life cycle - from germination and early growth to vegetative development and reproduction (Yan et al., 2020). The water deprivation conditions impose the plants not only cellular dehydration but also oxidative and heat stress, therefore interfering with a wide range of physiological and biochemical processes, including shoot and root architecture, biomass production; leaf area, leaf life-span and canopy size, photosynthesis, transpiration; nutrient acquisition, sink-source allocation, flowering, pod formation and seed maturity (Seleiman et al., 2021; Sato et al., 2024). There are strong lines of evidence on the tight correlation between soybean performance and severity of drought stress, with a higher yield loss under more prolonged water deficit conditions (Wei et al., 2018). Moreover, the drought impact on soybeans varies depending on the stage of the soybean life cycle that experiences with the adverse conditions. Previous findings demonstrated that water shortage during soybean reproduction might lead to a lower plant productivity by 24−82%, whereas a reduction of yield by 10−33% might be observed if the incidence takes place at the vegetative stage of soybean development (Brevedan & Egli, 2003; Wei et al., 2018).

Numerous studies have been conducted to elucidate the mechanisms underlying drought tolerance in plants, utilizing both mutant and overexpression plant systems. These investigations have highlighted the crucial regulatory roles of phytohormones (Chhaya et al., 2021; Das et al., 2025). Among the participants, cytokinins (CKs) have been identified as an important mediator of plant responses to water stress by regulating CK-responsive pathways (Nguyen et al., 2021). In general, CK metabolism is governed by the balance of CK synthesis and CK degradation, whereby Isopentenyl transferases (IPTs) are rate-limiting enzymes for the former process (Sakakibara, 2006). In addition, modulating the CK levels through *IPT* gene expression has been explored as a promising strategy to improve drought stress tolerance in several crop species, including rice (Peleg et al., 2011; Reguera et al., 2013), peanut (Qin et al., 2011), canola (Kant et al., 2015), cotton (Kuppu et al., 2013), maize (Bedada et al., 2016; Muruo et al., 2023), and wheat (Joshi et al., 2019; Beznec et al., 2021; Wang et al., 2023). However, such *in planta* characterization has yet to be conducted in soybean.

IPT-transgenic plants have been reported to exhibit improved drought tolerance and growth under stress conditions, characterized by more robust shoot and/or root morphology, delayed leaf senescence, better maintenance of pigment contents and photosynthetic activities, enhanced antioxidative capacity, increased water use efficiency, and/or more effective sink-source distribution (Nguyen et al., 2021). With the successfully developed transgenic plants thus far, stress-inducible promoters were commonly used to minimize the expression of *IPT* transgene under non-stressed growth conditions, thus avoiding the unnecessarily excessive production of CK levels leading to interference with normal plant growth and tissue development (Li et al., 1992; Bedada et al., 2016).

In the soybean genome, 14 members of the *IPT* gene family have been identified and annotated, based on homology to the *IPT* gene profile in *Arabidopsis thaliana* (Le et al.,

2012b). A comprehensive expression analysis of these genes under normal and water deficit revealed a subset of *IPT* members that were responsive to water deficit. Among these, *GmIPT10* (*Glyma07g38620.1*) has been shown to have significantly altered expression between the two conditions (Le et al., 2012b), prompting further investigation into its role in plant responses to drought stress. To characterize the drought-related function of *GmIPT10 in planta*, we selected soybean as the transformation system - a crop in which *IPT*-based biotechnological modulation remains explored. The *GmIPT10* transgene was placed under the control of the *Arabidopsis* drought-inducible *RD29A* (*Responsive to desiccation 29A*) promoter, which has been previously shown to function effectively in soybeans (Bihmidine et al., 2012). Following drought stress application at both vegetative and reproductive stages, *GmIPT10*-transgenic soybeans displayed better growth, enhanced photosynthetic performance and elevated antioxidant enzyme activities (Hoang et al., 2025). Therefore, in this study, we further assessed the drought tolerant capacity of the transgenic plants based on common stress indicators and pod-related traits.

MATERIALS AND METHODS

Plant materials

In this study, the soybean cultivar Williams 82 (W82), kindly provided by the RIKEN Institute (Japan), was used as both the wild-type control (WT) and the recipient for genetic transformation. *GmIPT10* was first cloned into the pGKX vector following a protocol of Qin et al. (2008). After that, the construct was transferred into pENTR Directional TOPO and finally recombined into the pTF101.1 gw1 binary vector. The target gene was placed under the control of the *RD29A* promoter and the $P_{35S::Bar}$ cassette was included for the selection of transformed plants. Soybean transformation mediated by *Agrobacterium* was conducted by an external service provider (Iowa State University, USA).

The resulting T_o seeds were self-pollinated over three successive generations. Segregation ratios of herbicide-resistant to herbicide-sensitive phenotypes were monitored to identify homozygous transgenic events, following Mendelian inheritance principles (Tizaoui & Kchouk, 2012). In this research, two confirmed, independent homozygous lines, L25 and L27, were used as the experimental materials for analyses.

Plant growth and drought stress assay

Soybean seeds were initially germinated at a density of three plants per pot, and seedlings were later thinned to retain only one per pot. Each container has a diameter of 10 cm, while the height varies depending on the stage of sample collection: 40 cm for vegetative-stage analyses and 80 cm for reproductive-stage analyses (Thu et al., 2014). Plants were grown under net-house conditions (28−33 ^oC, 60−70% relative humidity, natural photoperiod) and watered daily. Drought stress was imposed to the plants by water-withholding until the soil moisture dropped below 40%, starting at either the V1-V2 (early vegetative) or R1 (early reproductive) stage of soybean development (Thu et al., 2014; Wei et al., 2018). Leaf samples were collected at the end of the stress period for analysis of drought stress indicators.

For gene expression analysis, twelve-day-old plants were carefully uprooted and gently cleaned to remove adhering soil. The plants were then subjected to dehydration under laboratory conditions for 6 h (Le et al., 2012b). Non-dehydrated plants were collected and used as controls.

Measurement of malondialdehyde (MDA) contents

To evaluate the degree of membrane lipid peroxidation in both non-stressed and drought-stressed plants, MDA contents were analyzed using the thiobarbituric acid (TBA) assay following previous studies (Demirel et al., 2020) with modification. Briefly, 200 mg of the leaf tissue was homogenized in 1 mL of

0.1% trichloroacetic acid (TCA). After centrifugation at low temperature conditions, the supernatant was collected and mixed with 20% TCA solution containing 0.5% TBA in a 9:10 (v/v) ratio (supernatant: reaction solution). The mixture was then incubated at 95 °C for 15 min, then rapidly cooled in an ice bath for 10 min and centrifuged again. The absorbance of MDA and non-specific contents from the mixture were measured at 532 nm and 600 nm wavelengths, respectively, using the reaction solution as a blank. The MDA content was calculated using the following formula: MDA (µmol/g FW) = $[(A_{532} - A_{600}/\varepsilon] \times 10^3 \times$ dilution factor × [1/tissue weight (g)], where ε (extinction coefficient) = 155 $mM^{-1}cm^{-1}$. For this assay, four biological replicates per treatment were used for the analyses.

Measurement of cellular ion leakage

Membrane ion leakage was evaluated following the methods of Kim et al. (2018) with modifications. Eight leaf discs (0.8 cm in diameter) per replicate were used for the assay. The discs were incubated in 25-mL Milli-Q water for 24 hours at room temperature conditions and the initial conductivity (C_i) of the samples was measured using a digital conductivity probe (EC/TDS HI763100, Hanna Instruments, Romania). The samples were next heated at 95 ^oC for 30 min. Once cooled to room temperature, each sample was re-measured to obtain the maximal conductivity (C_{max}). Five biological replicates were used per treatment. Conductivity of Milli-Q water (C_o) without sample was also measured for normalization. The percentage of cellular ion leakage was calculated using the following formula: Relative ion leakage (%) = $(C_i - C_o)/(C_{max} - C_o) \times 100$.

Gene expression analysis

To examine the expression of drought-related genes by reverse transcription quantitative PCR (RT-qPCR), leaf tissues were collected from plants subjected to 0-h and 6-h-dehydration treatments and ground into fine powder in liquid nitrogen. Total RNA was isolated using the GeneJET Plant RNA Purification Kit and the RapidOut DNA Removal Kit (Thermo Fisher Scientific, USA). The RNA quantity and purity were assessed using a Nanodrop spectrophotometer (Biochrom, USA). For reverse transcription, complementary DNA (cDNA) was synthesized in 20-µL reactions using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA), with 1,000 ng total RNA per sample. For qPCR, 1 uL of cDNA was used in 25-µL reactions prepared according to the manufacturer's instruction, with primer concentrations of 0.4 µM (Maxima SYBR Green qPCR Master Mix, Thermo Fisher Scientific, USA). The thermal cycling condition was based on the study of Thao et al. (2013), followed by a melting curve analysis. The sequences of primers used to analyze gene expression are listed in Table 1. The house-keeping gene *GmFbox* was used as the reference for normalization in relative expression analyses, using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001; Le et al., 2012a) (Table 1). The qPCR analysis was conducted with biological triplicates.

Analyses of yield-related components

Following the imposition of water stress on WT and transgenic lines at the flowering (R1) stage until the soil moisture declined below 40%, the plants were re-irrigated normally until pod harvest (n = 12). The collected pods were grouped into seedless pod, pods containing one, two, three or four seeds, and the percentage of each pod category was calculated. Non-stressed plants were included as controls.

Data analyses

The data collected during the study were presented as the mean ± standard error (SE) of replicates. Student's t-test was used to determine statistically significant differences ($p < 0.05$) either between a transgenic line and the WT under the same condition, or between different conditions for the same genotype.

Table 1. Primer sequences used in RT-qPCR

Gene	Primer sequence (5'-3')	Amplicon size (bp)	References
GmRD20A (*Response to desiccation 20A*)	Left: GTGGCACATGACTGAAGGAA Right: ATCTTTCCAGCAGCACCTCT	195	Neves-Borges et al. (2012)
GmERD1 (*Early response to dehydration 1*)	Left: CGTCCAGAATTGCTCAACAG Right: TGGGGTTATAGCCTTGTTGG	184	
GmPOD (*Peroxidase*)	Left: ACTCTCAGGTGGTCATACG Right: ATCAGGTGTGCTCAGGTC	187	Jiao et al. (2016)
GmCAT (*Catalase*)	Left: CTGCTGGAAACTATCCTGAGTG Right: ATTGACCTCTTCATCCCTGTG	395	Zilli et al. (2009)
GmGST4 (*Glutathione S-transferase 4*)	Left: GTTCCATTCTACACTTGGTTC Right: ACCACTACTTCACAATGGCC	222	Dalton et al. (2009)
GmSGR1 (*Stay-green 1*/*Non-yellowing 1*)	Left: TCCACAGCCATGCCAAGAAG Right: AGCATCCCCGTAACTGTGAAT	171	Shi et al. (2016)
GmFbox	Left: CTAATGGCAATTGCAGCTCTC Right: AGATAGGGAAATTGTGCAGGT	93	Le et al. (2012a)

RESULTS

Plant stress status *via* assessment of drought-associated stress indicators

The MDA assay is a widely used method for assessing the extent of lipid peroxidation in cell membranes, which results from the attack of reactive oxygen species (ROS) to lipid molecules (Zhang et al., 2021), whereby elevated MDA levels indicate more severe membrane damage (Hasanuzzaman et al., 2021). In this study, two independent *GmIPT10*-transgenic lines (L25 and L27) were used for comparison with the non-transformants. Our results showed that under normal conditions, both WT and transgenic plants exhibited low and comparable MDA contents at the vegetative (V) (Fig. 1a) and reproductive (R) stages (Fig. 1b). Under drought stress, MDA levels increased in all genotypes; however, the increase was substantially higher in the WT plants. Compared to their respective well-watered controls, MDA contents in the stressed WT plants increased approximately 3.5-fold at the V stage and 2.4-fold at the R stage. In contrast, the transgenic plants exhibited lesser increases: 1.82-fold and 1.79-fold in line L25, and 2.34-fold and 1.3-fold in line 27, respectively (Figs. 1a, b). These results indicate that the drought-treated WT plants accumulated significantly more MDA, with levels approximately 1.4- to 2.0-fold higher than those observed in the stressed transgenic lines.

A similar trend was also observed in electrolyte leakage analysis, a widely accepted indicator of plasma membrane integrity, thus a reliable parameter for evaluating plant stress-induced injury and stress tolerance. When the living membrane is damaged, higher levels of ions, including cations and anions, can leak out of the cell in an uncontrolled manner (Demidchik et al., 2014). As shown in Figures 1c & 1d, the degree of ion leakage in the WT plants increased markedly from around 10% under well-watered conditions to 63% at the V stage and even higher (77%) at the R stage following drought treatment. In contrast, the transgenic lines maintained relatively stable ion leakage under non-stressed conditions (~10%) and showed only moderate increases under drought stress, ranging from 28% to 42% at the V stage and from 45% to 49% at the R stage.

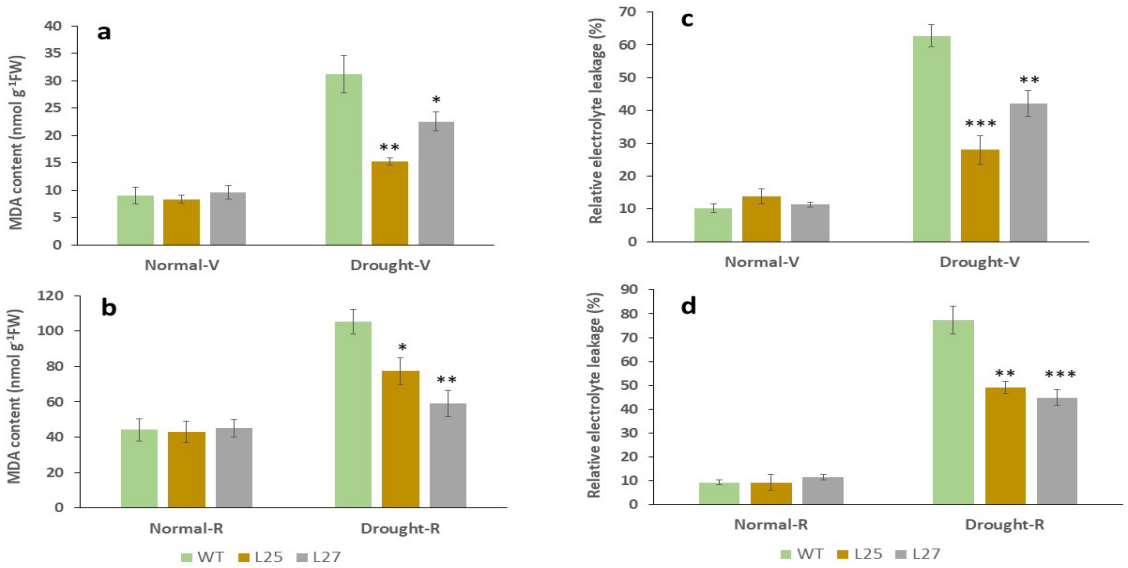

Figure 1. Drought stress evaluation for wild-type (WT) and two *IPT*-transgenic soybean lines (L25 and L27) based on physiological stress indicators. a. Malondialdehyde (MDA) contents in leaf tissues under well-watered and drought-stressed conditions at the vegetative (V) stage. b. MDA contents in leaf tissues under well-watered and drought-stressed conditions at the reproductive (R) stage. c. Electrolyte (ion) leakage from leaf tissues under well-watered and drought-stressed conditions at the V stage. d. Electrolyte leakage from leaf tissues under well-watered and drought-stressed conditions at the R stage. Significant differences between each transgenic event and WT under the same condition, as determined by Student's *t*-test, are indicated by asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

Effects of drought on pod formation and filling

Water availability is an important abiotic factor influencing plant growth, development and productivity, particularly during the reproductive period (Wei et al., 2018; Dietz et al., 2021). In light of previous findings, this aspect was also explored in this study to further investigate the potential contribution of the *IPT* transgene in mitigating yield loss.

According to the pod analysis, the pod profiles of transgenic and WT plants were similar under irrigation conditions. Although slight differences were observed in the proportions of pods containing one, two or three seeds between genotypes, these variations were not statistically different. The majority of pods contained either two seeds (60% in WT vs. 52%−58% in transgenic lines) or three seeds (31% in WT vs. 28%−37% in lines L25 and L27) (Fig. 2). Meanwhile, drought stress imposed at the R1 stage has remarkably altered the pattern of pod development in the WT group. Specifically, the percentage of single-seeded pods increased significantly from 6% (under normal conditions) to 23%, while the proportion of three-seeded pods declined threefold to just 10% (Fig. 2). In contrast, the transgenic plants could maintain a high degree of pods bearing two or more seeds, accounting for an average of 84% of total pods produced per plant in transgenic line L25 and 77% in line L27. Consequently, under the adverse conditions, the transformants displayed a considerably lower proportion of one-seeded pods (L25) and a higher percentage of three-seeded pods (L25 and L27) compared with their non-transgenic control (Fig. 2). It has been also noted that drought led to an increase in the proportion of pods with immature or undeveloped seeds, classified as seedless, by 19% (in the WT

plants), whereas the increase was limited to 5%-10% in the transgenic lines. These findings suggest that the transgenic plants possessed a greater capacity to sustain seed development under drought, which contributes to higher yield potential.

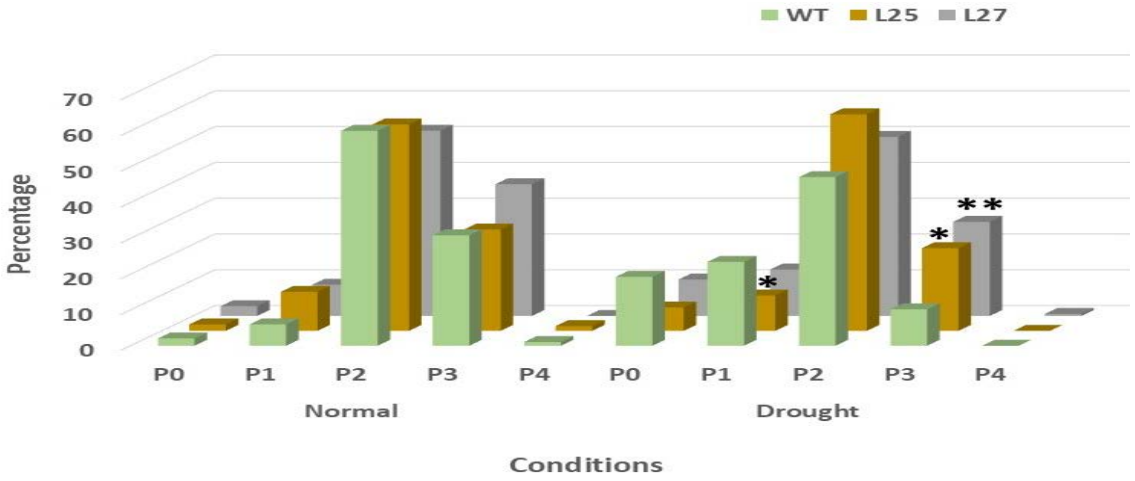

Figure 2. Pod analysis of wild-type (WT) and two *IPT*-transgenic soybean lines (L25 and L27) under well-watered and drought stress conditions applied at the R1 stage (n = 12). The proportion of pods without seeds (P0), having one (P1), two (P2), three (P3) and four (P4) seeds are presented. Significant differences between each transgenic event and WT under the same condition, as determined by Student's *t*-test, are indicated by asterisks (* $p < 0.05$; ** $p < 0.01$)

Effects of drought on the expression of drought-responsive genes

In addition to biochemical and pod traits analyses, transgenic line L25 was selected for further investigation on the expression of several key drought-related genes to gain insights into the underlying molecular responses. Expression analyses of two marker genes, *GmRD20A* (*Responsive to desiccation 20A*) and *GmERD1* (*Early responsive to dehydration 1*), revealed strong induction after 6 hours of dehydration treatment. Notably, transcript levels were greatly higher in the transgenic plants than in the WT plants under stress conditions, with *GmRD20A* and *GmERD1* showing 1.6-fold and 1.4-fold higher, respectively (Figs. 3a, b).

Among the various plant responses to water scarcity, enhanced expression and activity of antioxidant enzymes is a crucial defensive mechanism. These enzymes, located in the cytosol and various organelles, are mainly responsible for the production of excessive ROS due to their disrupted activities under drought stress conditions (Dumanovíc et al., 2021). In this study, two important genes, *GmPOD* and *GmCAT*, which encode hydrogen peroxide-scavenging workhorses, were examined for their expression. The analyses showed that both genes were upregulated under water stress conditions. However, the dehydrated-transgenic tissues exhibited more pronounced transcript levels of both *GmPOD* and *GmCAT* (by 1.8-2.5-fold), whereas the stressed WT plants showed only a slight increase in the *POD* expression (Figs. 3c, d).

GST genes encode glutathione S-transferases, another subset of antioxidant enzymes that catalyze ROS detoxification, such as lipid peroxides, using glutathione as a reducing power (Dalton et al., 2009). Consistent with the greater upregulation of *GmPOD* and *GmCAT* under dehydration conditions, the transgenic plants also acquired higher expression of *GmGST4* by approximately 1.4-fold compared with the dehydrated WT plants, and by 5.8-fold

compared with their non-stressed counterparts (Fig. 3e). In contrast, expression of *GmSGR1* (*Stay-Green 1*/*Non-yellowing 1*), a key enzyme working in the chlorophyll catabolic pathway (Shi et al., 2016; Yamatani et al., 2021), was induced by dehydration in both genotypes, yet at a lesser extent in the transformant (Fig. 3f).

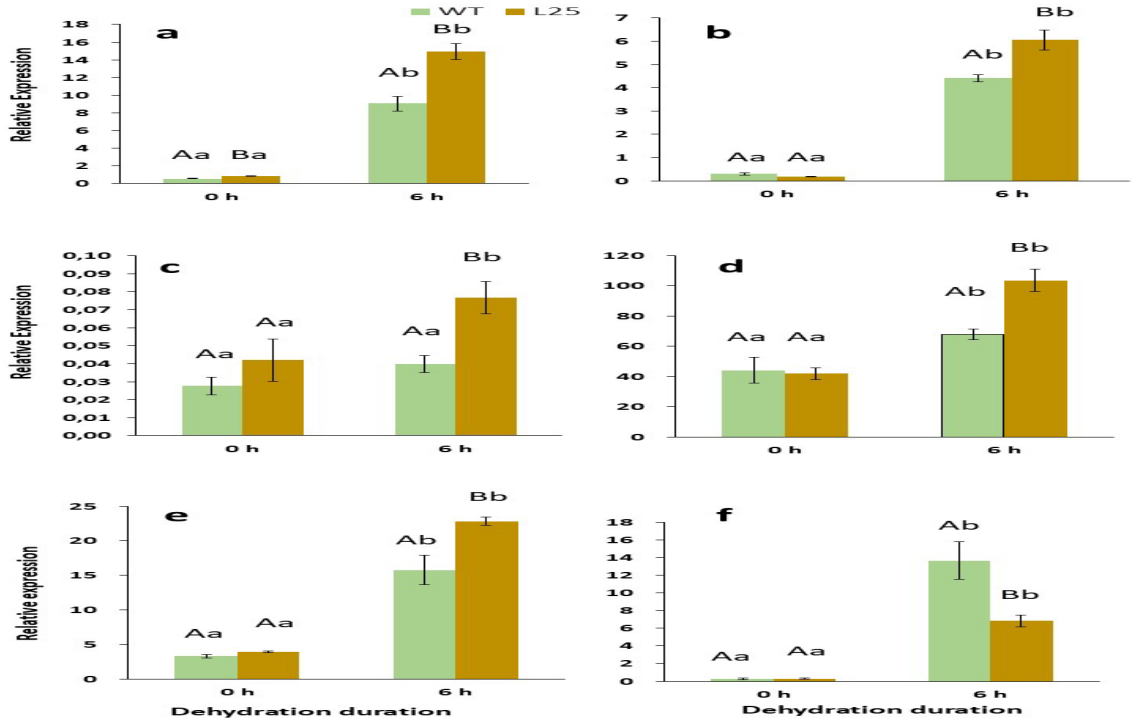

Figure 3. Dehydration stress evaluation for wild-type (WT) and *IPT*-transgenic soybeans (L25) based on analyzing gene expression that are related to drought stress. a. Expression of *GmRD20A*. b. Expression of *GmERD1*. c. Expression of *GmPOD*. d. Expression of *GmCAT*. e. Expression of *GmGST4*. f. Expression of *GmSGR1*. The leaf tissues were used for RT-qPCR. Different capital letters indicate a statistically significant difference between genotypes under the same conditions. Different lowercase letters indicate statistically significant differences between conditions for the same genotype, as determined by Student's *t*-test

Taken together, these findings highlight that the *IPT*-transgenic soybeans likely experience less injuries caused by drought-induced oxidative stress, potentially due to enhanced antioxidant defense mechanisms.

DISCUSSION

According to our previous analyses, *GmIPT10*-transgenic lines showed more vigorous growth, stronger antioxidant enzyme activities, including peroxidase (POD) and catalase (CAT), as well as more sustained photosynthetic performance under water stress conditions compared to WT plants (Hoang et al., 2025). Therefore, in this study, we further evaluated the drought tolerance of these transgenic lines by examining additional drought-associated parameters to obtain a more comprehensive understanding of these responses. Drought and dehydration stress conditions, which also triggers oxidative stress as a secondary negative effects, lead to the accumulation of various endogenous ROS such as hydrogen peroxide, singlet oxygen, superoxide, hydroxyl, and peroxyl radicals (Zhou et al., 2020). Our findings suggest the transgenic plants experience a lesser degree of drought-induced oxidative stress, as indicated

by their lower MDA contents coupled with lower levels of ion leakage (Fig. 1), which reflect reduced oxidative damage and maintained membrane stability. It has been reported that when the membrane is no longer intact, the efflux of K^+, a macronutrient, is intensified (Demidchik et al., 2014). This can trigger the alongside movement of other ions out of the cell, causing the imbalance in endogenous concentrations of the ion component profile. Additionally, it is reported that although moderate K^+ loss under mild stress conditions can stimulate plant immune response and adaptation, prolonged or severe stress with excessive cellular potassium ion depletion may promote program cell death, due to activating protease and nuclease enzymes (Demidchik et al., 2014).

From previous studies, there are strong lines of evidence demonstrating a correlation between *IPT* expression, drought tolerance and levels of MDA and/or electrolyte leakage. In wheat, knockout of *TaIPT8* led to reduced drought tolerance, which was associated with substantially higher MDA accumulation in the mutant lines (Wang et al., 2023). In a more recent study, *IPT*-transgenic ryegrasses - driven by the stress-inducible *RD29A* promoter - exhibited markedly lower electrolyte leakage percentage and MDA levels compared with the non-transgenic counterparts under drought conditions. Moreover, post-drought recovery analyses of these parameters further suggested better cell stability and resilience in the transgenic lines, as their ion leakage and MDA contents returned to levels comparable or even lower than those of well-watered controls. In contrast, the drought-treated WT failed to show such recovery (Esmaeili et al., 2025).

According to gene expression analysis, the transgenic plants acquired stronger upregulation of *GmRD20A* and *GmERD1* in response to drought stress (Figs. 2a, b). These genes are homologs of *RD20A* (*AT2G33380*) and *ERD1* (*AT5G51070*) in *Arabidopsis*, which are ubiquitous marker genes for plant acclimation to drought stress and known to function in abscisic-dependent and abscisic-independent pathways, respectively (Nerves-Borges et al., 2012; Chen et al., 2021). As RD20 protein contains a calcium ion- binding domain, its work is hypothesized to be involved in Ca^{2+}-mediating plant responses to drought (Takahashi et al., 2000). It has been noted that, under well-watered conditions, the transgenic plants also exhibited significantly higher *GmRD20A* expression than WT, possibly due to leaky expression of the transgene. This basal upregulation may contribute to a primer state, enabling the plant preparation to respond to the forthcoming stress. Moreover, previous comparative analyses of *ERD1* expression between drought-tolerant versus drought-sensitive soybean genotypes also showed higher upregulation in both root and leaf tissues of the former groups, emphasizing role of ERD1 in mediating plant stress tolerance (Nguyen et al., 2024).

Our results also highlighted the consistency with previous reports, showing that both *POD* and *CAT* genes were upregulated under water stress conditions (Figs. 3c, d) (Rivero et al., 2007; Laxa et al., 2019; Chuong et al., 2021). Previously, it has been found that *Arabidopsis* plants subjected to 12 days of drought displayed strong catalase activity, but peroxidase activity was not significantly affected (Hoang et al., 2020). This discrepancy may be attributed to the differential timing of CAT and POD activation during drought progression. Additionally, as soybeans contain a large and diverse *POD* multigene family with over 100 members, their expression may vary depending on the intensity and duration of drought stress (Aleem et al., 2022). Therefore, it is important to investigate the expression profiles of other *POD* isoforms to identify specific members that are responsive to particular stress conditions. In addition to POD and CAT, GSTs represent another important class of antioxidant enzymes that are responsible for facilitating the removal of lipid peroxidation end products. Therefore, the elevated *GmGST* expression observed in the *GmIPT10*-transgenic plants (Fig. 3e) implies higher GST activities, which may contribute to more efficient mitigation of oxidative damage,

particularly the protection of membrane integrity from ROS-induced injury.

Meanwhile, the differential expression of *GmSGR1* between line L25 and WT plants, with significantly lower transcript abundance in the former group (Fig. 3f), suggests a lower degradation of photosynthetic pigments in the transgenic plants under the adverse conditions. This trait likely contributes to improved photosynthesis performance in the *GmIPT10*-transgenic plants. GmSGR1 is a key enzyme working in the chlorophyll catabolic pathway; thus, its activity also influences the onset of leaf senescence as well as overall photosynthetic performance in plants (Shi et al., 2016; Yamatani et al., 2021).

The drought stress can negatively impact yield components in different ways, reducing both the quantity to quality of the harvest (Dietz et al., 2021). Previous results have shown that drought has a more pronounced effect on pod filling and seed development than on the total number of pods produced (Chen et al., 2021; Amjid & Üstün, 2025). In our study, transgenic soybean lines harboring *GmIPT10* could maintain better capacity of pod filling and development under drought stress conditions, indicated by substantially higher proportion of pods containing multiple seeds and a lower percentage of seedless pods (Fig. 2). These findings also align with previous studies which emphasize a positive correlation between yield stability and drought resilience (Chen et al., 2021; Qin et al., 2024).

Successful enhancement of productivity under drought conditions has been reported in various transgenic crops expressing the *IPT* gene, including canola (Kant et al., 2015), sweet potato (Nawiri et al., 2018), wheat (Beznec et al., 2021) and maize (Muruo et al., 2023). In general, limited access to water resources during the flowering stage can shorten the flowering period and promote flower abortion, thus collectively leading to a reduction in flower number and a lower rate of successful pod formation (Amjid & Üstün, 2025). Meanwhile, one of the crucial regulatory roles of CKs is to support reproduction by facilitating complete gametogenesis, preventing flower and pod abortion, enhancing biomass accumulation and nutrient deposition during seed development (Bedada et al., 2016; Beznec et al., 2021). Therefore, increasing CK contents through *IPT* gene expression can offer distinct advantages to engineered plants by mitigating drought-induced reproductive failure and contributing to improved yield performance.

Taken together, our results demonstrate that *GmITP10*-transgenic soybeans exhibit enhanced drought tolerance, which can be at least attributed to the more effective mitigation of drought-induced oxidative stress effects and the prevention of photosynthetic pigment degradation, thereby supporting improved reproductive performance.

CONCLUSION

The findings from this study highlight the beneficial potential of *GmIPT10* in enhancing plant adaptation to water scarcity conditions. Under the stress conditions, the transgenic soybean plants exhibited reduced cellular membrane damage, likely resulting from enhanced antioxidant defense mechanisms, as well as maintained higher seed yield. These results warrant further in-depth investigations to elucidate the comprehensive biological functions of *GmIPT10* in mediating plant tolerance towards drought stress.

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