agronomy

MDPI

Article

Potato E3 Ubiquitin Ligase *StXERICO1* Positively Regulates Drought Resistance by Enhancing ABA Accumulation in Potato and Tobacco and Interacts with the miRNA Novel-miR1730-3p and Proteins StUBC and StTLP

Jing Yi [1], Lai Wang [1], Yongkun Chen [1,2], Canhui Li [3] and Ming Gong [1,*]

[1] School of Life Sciences, Yunnan Normal University, Southwest United Graduate School, Key Laboratory of Potato Biology of Yunnan Province, Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Ministry of Education, Key Laboratory of Biomass Energy and Environmental Biotechnology of Yunnan Province, Kunming 650500, China; yijing@ynnu.edu.cn (J.Y.); 2123120001@ynnu.edu.cn (L.W.); chenyk@xjnu.edu.cn (Y.C.)

[2] Xinjiang Key Laboratory of Special Species Conservation and Regulatory Biology, Key Laboratory of Plant Stress Biology in Arid Land, School of Life Sciences, Xinjiang Normal University, Urumqi 830054, China

[3] Yunnan Key Laboratory of Potato Biology, Joint Academy of Potato Science, Yunnan Normal University, Kunming 650500, China; 263@ynnu.edu.cn

* Correspondence: gongming@ynnu.edu.cn

Abstract: Potato (*Solanum tuberosum* L.) is sensitive to drought, which severely impacts tuber yield and quality. In this study, we characterized a *XERICO* gene, encoding a RING-H2 type E3 ubiquitin ligase, *StXERICO1*, from a diploid potato, investigated its role in enhancing drought resistance and ABA accumulation, and identified its interaction with the miRNA novel-miR1730-3p, as well as its protein interactions with StUBC and StTLP. *StXERICO1*, with a complete Open Reading Frame (ORF) of 459 bp encoding 152 amino acids, was highly responsive to drought, ABA treatment, and abiotic stresses in potato plants. Overexpression of the *StXERICO1* significantly enhanced drought resistance and ABA accumulation in transgenic potato and tobacco plants and exhibited greater sensitivity to ABA treatment, which was associated with the upregulation of expression of ABA biosynthetic genes *NCED* and *CYP707A*. Furthermore, our results revealed that *StXERICO1* and its encoding protein interacted with miRNAs and other proteins. 5′ RLM-RACE (cDNA terminal rapid amplification) experiment showed that the miRNA novel-miR1730-3p targets 5′ UTR region of the *StXERICO1* gene. Dual luciferase assay and virus-based miRNA silencing experiment showed that the novel-miR1730-3p negatively regulates *StXERICO1* expression. Moreover, yeast two-hybrid assay indicated that StXERICO1 interacts with StUBC (an E2 ubiquitin ligase) and StTLP (a Tubby-like protein), suggesting that StXERICO1 might function on ABA homeostasis at the post-translational level. These findings elucidate the molecular mechanisms by which StXERICO1, a RING-H2 type E3 ubiquitin ligase, enhances drought resistance through increased ABA accumulation, how its expression is regulated by miRNA, and how it exerts its function through interactions with other proteins. The results also provide a potential candidate gene for subsequent precision molecular breeding aimed at improving crop drought resistance.

Keywords: potato (*Solanum tuberosum* L.); *StXERICO1*; drought tolerance; abscisic acid; miRNA

Citation: Yi, J.; Wang, L.; Chen, Y.; Li, C.; Gong, M. Potato E3 Ubiquitin Ligase *StXERICO1* Positively Regulates Drought Resistance by Enhancing ABA Accumulation in Potato and Tobacco and Interacts with the miRNA Novel-miR1730-3p and Proteins StUBC and StTLP. *Agronomy* **2024**, *14*, 2305. https://doi.org/10.3390/agronomy14102305

Academic Editor: Jadwiga Śliwka

Received: 19 September 2024
Revised: 2 October 2024
Accepted: 4 October 2024
Published: 7 October 2024

1. Introduction

Extreme weather is one of the primary factors limiting global crop yields. Between 1964 and 2007, global droughts and high-temperature events led to a 9–10% reduction in cereal production [1]. Due to extreme drought conditions, which have now changed to a compound of dry-hot conditions, there is a possibility that maize yield reductions may increase from 7% to 31% in the future [2]. Potato (*Solanum tuberosum* L.), ranking as the third

most consumed crop globally following rice and wheat, is considered drought-sensitive and susceptible to yield loss due to drought stress. Assessing the future climate change impacts on potato production in Prince Edward Island, Canada, reveals that by the 2070s, potato yields are expected to decline by 48% to 60%, with even more significant drops of 63% to 80% projected by the 2090s [3].

Protein ubiquitination, a post-translational modification, plays a crucial role in regulating protein function and stability in eukaryotic cellular activities [4,5]. This modification also plays key roles in plant growth, development, and both biotic and abiotic stress responses and adaptation by regulating the abundance, activity, or subcellular localizations of various regulatory polypeptides and enzymes. The process of protein ubiquitination involves the sequential actions of ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin ligase (E3) enzymes. Importantly, diverse E3 ligases are involved in these regulatory pathways, mediating phytohormone and light signaling or other pathways [6,7]. In particular, RING (Really Interesting New Gene) E3 ligases are considered key regulatory components involved in plant responses to abiotic stresses [8]. The distinction between the two canonical RING domain proteins, RING-HC (C3HC4) and RING-H2 (C3H2C3), is based on the presence of His and Cys at the fifth metal ligand position, respectively [9]. The RING-H2 gene family is the most abundant type of RING-type E3 ubiquitin ligases, with a total of 241 members reported in *Arabidopsis*, 249 in *Populus*, and 292 in *flax* [10–12].

MicroRNAs (miRNAs) are non-coding RNAs that are widespread in eukaryotes, typically 20 to 24 nucleotides in length. In plants, miRNAs primarily regulate gene expression negatively by cleaving mRNA or inhibiting translation, participating in the regulation of various biological processes such as plant morphogenesis, cell differentiation, tissue formation, metabolism, growth and development, hormones, signal transduction, and response to various stresses [13–15]. Research has shown that drought stress can stimulate the production of multiple miRNAs in plants and alter their expression. These miRNAs participate in forming a complex gene regulatory network to cope with and adapt to drought stress by interacting with specific target genes. Therefore, miRNAs play a crucial role in the plant's response to drought stress [16,17].

XERICO (Greek for 'drought tolerant'), a RING-type E3 ubiquitin ligase gene, encodes a small protein with 162 amino acids that can regulate abscisic acid (ABA) levels and promote drought tolerance when overexpressed in *Arabidopsis thaliana* [18]. There were two RING-H2 genes, *ZmXERICO1* and *ZmXERICO2*, in maize (*Zea mays*), and the overexpression of these genes conferred improved drought tolerance in both *Arabidopsis* and maize [19]. A RING-H2 zinc finger from *P. trichocarpa* is identified as the closest homolog of Arabidopsis *XERICO*, and overexpression of *PtXERICO* confers an enhanced drought stress tolerance in *Arabidopsis* and poplars [20]. CBF4/DREB1D represses *XERICO* to attenuate ABA, osmotic, and drought stress responses in *Arabidopsis* [21]. However, there is no evidence yet regarding whether and which miRNAs are involved in the regulation of *XERICO* gene expression.

Based on our previous RNA-seq results in potato plants during drought-rehydration cycles [22], we found that a potato *XERICO* gene was highly responsive to drought stress and exhibited differential expression under drought and re-drought treatments compared to the control and rehydration treatments. In this study, we cloned and characterized *StXERICO1* from a diploid potato and analyzed its expression pattern using RNA-seq and qRT-PCR. We also investigated the effects of overexpression and CRISPR/Cas9-induced mutation on drought resistance, ABA accumulation, and the potential underlying mechanisms in both potato and tobacco plants. Through 5′ RLM-RACE, dual luciferase assay, and virus-based microRNA silencing experiments, we verified that *StXERICO1* is a target molecule of novel-miR1730-3p. In addition, a yeast two-hybrid assay revealed that StXERICO1 protein interacts with StUBC (an E2 ubiquitin ligase) and StTLP (a Tubby-like protein). These findings provide insights into the molecular mechanisms of *StXERICO1* in enhancing drought resistance and ABA accumulation, as well as its regulation by miRNAs

and protein interactions. The results also identify *StXERICO1* as a potential candidate gene for future molecular breeding aimed at improving crop drought resistance.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The diploid clones CIP 703541 of *Solanum tuberosum* group Phureja and CIP706205 (*Solanum ajanhuiri* Juz. & Bukasov, Jancko Sisu Yari) of *Solanum tuberosum* group Ajanhuiri from the International Potato Center (CIP), along with *Nicotiana benthamiana* and *Nicotiana tabacum* cv. Xanthi from the Research Section of Stress Biology of Plants, Yunnan Normal University, were used in this study. The plants were grown on soil in a growth chamber or on solid Murashige and Skoog (MS) medium (pH 5.8) containing 3% (*w*/*v*) sucrose in a growth room at 24 ± 1 °C under 16-h light/8-h dark cycles.

2.2. Cloning and Sequence Analysis of StXERICO1 Gene

The full-length *StXERICO1* cDNA was cloned from the cDNA of *Solanum tuberosum* CIP706205. The amplified fragment was ligated into a cloning vector using the pBM16A Topos-mart Cloning Kit (Biomed, Beijing, China) and sequenced at Sangon Biotech to ensure accuracy. The *StXERICO1* gene was translated into an amino acid sequence using DNAMAN version 9.0 software. Using online tools Protparam (https://web.expasy.org/protparam/, accessed on 31 March 2022), we analyzed the physical and chemical properties of the StXERICO1 protein. The homology of the StXERICO1 protein was analyzed using BLASTP in the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 31 March 2022). Multiple sequence alignment and phylogenetic analysis were performed using both DNAMAN version 9.0 software and MEGA version 7.0 software. The neighbor-joining (NJ) method was used to construct the phylogenetic tree with 1000 bootstrap replicates, and default parameters were applied for the remaining settings. All primers used in this assay were listed in Supplementary Table S1.

2.3. RNA Extraction and Gene Expression Patterns Analysis

The potato variety CIP706205 (*Solanum ajanhuiri* Juz. & Bukasov, Jancko Sisu Yari) was used as the experimental material. After rooting in tissue culture, the plantlets were transplanted into pots containing a mixed substrate and grown for one month. Drought stress was induced by withholding irrigation, following the method described by Chen et al. [22]. When the leaves began to curl after a week of restricted watering, they were collected as samples for mild drought. Once the leaves became fully curled and wilted, they were collected as samples for severe drought. The potato plants were then re-watered. After one day of recovery, the curled leaves fully unfolded and were collected as samples for rehydration. The potato plants were subsequently subjected to a second dehydration treatment. When the same phenotypes reappeared, the leaves were collected as samples for mild re-drought and severe re-drought, respectively. The leaves from normally watered plants were collected as the control.

Total miRNA and RNA were extracted from samples under different treatments using the DP504 miRcute miRNA extraction kit (Tiangen, Beijing, China) and the DP441 RNAprep Pure Plant Kit (Tiangen, Beijing, China), respectively. First-strand cDNA was synthesized using the KR221 miRcute miRNA cDNA First-Strand Synthesis Kit (Tiangen, Beijing, China) and the RR407A Prime Script RT reagent Kit (TaKaRa, Beijing, China). Three biological replicates were used for each treatment. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis was conducted using TB Green® Premix Ex Taq TM II (TaKaRa, Beijing, China) on a LightCycler 960 (Roche Diagnostics, Basel, Switzerland). Three technical replicates were performed for each biological replicate. *U6* and *StEF1a* were used as internal controls to normalize the expression of miRNA and mRNA, respectively. The relative gene expression level was determined using the $2^{-\Delta\Delta Ct}$ method.

The transcriptome dataset of *StXERICO1* from our research was downloaded from Spud DB (https://spuddb.uga.edu/, accessed on 1 August 2022) and from the National Center

for Biotechnology Information Sequence Read Archive (SRA) database (accession numbers: PRJNA661171). The expression patterns of *StXERICO1* were analyzed across different tissues, various abiotic stresses, and different hormone treatments. Data processing was conducted using Excel. FPKM was used to analyze expression pattern data from different tissues, while $Log_2^{FoldChange}$ was applied to expression profile data under abiotic stress and hormone treatments. The processed data were visualized using TBtools software (v1.120).

2.4. Vector Construction for Overexpression and Knockout of StXERICO1 in Transgenic Plants

The coding sequence of the *StXERICO1* gene was amplified using a high-fidelity polymerase and then inserted downstream of the 35S promoter in the PEZR_(K)-LN plant expression vector using In-Fusion cloning techniques. *Agrobacterium* strain EHA105 was used to transform both potato (CIP 703541) and tobacco (*Nicotiana tabacum* cv. Xanthi). Potato was transformed using the stem transformation-regeneration method [23]. The regenerated plants were initially screened for growth on a selective medium supplemented with 50 mg·L^{-1} kanamycin. Due to the phenomenon of chromosome doubling in the potato stem transformation-regeneration method, the chromosome ploidy of the regenerated positive lines was detected using flow cytometry. Genomic DNA was extracted from each regenerated diploid plant for PCR amplification to confirm the presence of *StXERICO1*, while total RNA was isolated for expression analysis of *StXERICO1* using qRT-PCR. After verification, the lines were used in all subsequent experiments. Tobacco was transformed using the leaf disk transformation-regeneration method. Following regeneration and DNA/RNA validation, the T0 transgenic tobacco plants were self-crossed, and the resulting T1 seeds were used for all subsequent experiments.

The knockout of *StXERICO1* in potato was achieved using CRISPR/Cas9-mediated genome editing technology, as described in our recent publication [24]. In brief, the knockout vector targeting *StXERICO1* was constructed. The coding sequence of *StXERICO1* was analyzed using the CRISPR-P tool 2.0 online website (http://cbi.hzau.edu.cn/cgi-bin/CRISPR2/CRISPR, accessed on 2 March 2023), and a 20 nt single-guide RNA sequence was selected. The primer was annealed, diluted 10-fold, and then ligated onto the CRISPR/Cas9 knockout vector using the *Bsa* I restriction enzyme. Subsequently, the constructed vector was introduced into *Agrobacterium* strain EHA105 for the transformation of potato via the stem transformation-regeneration method [23]. Following this, the diploid lines were identified using flow cytometry. Genomic DNA was extracted from each regenerated diploid plant for PCR amplification. PCR amplicons were then cloned into the pBM16A vector (Biomed, Beijing, China), and 10 clones were sequenced to confirm the types of mutations at the target sites.

2.5. Assessment of Drought Resistance and ABA Content in Soil-Grown Transgenic Tobacco and Potato Plants under Drought Stress

Transgenic and wild-type (WT) potato plants were planted into pots containing a mixed substrate and grown for at least a month. Drought stress was applied by withholding irrigation. When the leaves of the plants were fully curled and wilted after approximately 16–17 days of drought treatment, the plants were re-watered.

Approximately 150 mg of each tissue sample was collected, flash-frozen in liquid nitrogen, finely ground to a powder, and stored at −80 °C until analysis. The extraction, purification, and quantification of ABA were performed following the method [25]. The supernatants were transferred to glass vials and were analyzed by HPLC-MS/MS (LCMS-8040, Shimadzu). Measurements were conducted using an LC-20AD liquid chromatography system (Shimadzu). Three replicated leaf samples were analyzed for each plant type.

2.6. Assessment of Growth in Transgenic Potato Plants under ABA Treatment and Normal Conditions

Thirty-day-old transgenic and wild-type (WT) potato plants grown in tissue culture were used for ABA treatment. Apical buds of similar length were placed on solid Murashige

and Skoog (MS) medium (pH 5.8) containing 15 μM and 20 μM ABA, respectively. After two weeks, the longest root of each of the three plants from each line was measured and photographed. In addition, we measured and photographed the shoot height and root length of transgenic lines and wild-type potato plantlets.

2.7. Prediction and Experimental Verification of Cleavage Site of StXERICO1 by miRNAs

The miRNA cleavage site of the *StXERICO1* gene was predicted using the bioinformatics website psRNATarget (https://www.zhaolab.org/psRNATarget/, accessed on 31 March 2023). The predicted miRNA cleavage site was experimentally confirmed through a 5′ RLM-RACE assay using the FirstChoice® RLM-RACE Kit (Thermo Scientific, Waltham, MA, USA). Specific primer sequences for the 5′ RLM-RACE were designed using Geneious software (version 9.02) as Supplementary Table S1. After nested PCR amplification, the PCR product was gel-purified, ligated with the pBM16A vector, and 10 clones were sequenced. Geneious V9.02 software was used to verify the availability of sequencing peak map results, followed by sequence alignment to determine the cleavage site and efficiency on the *StXERICO1* transcript by novel-miR1730-3p.

2.8. Dual Luciferase Assay in N. benthamiana Leaves

In this study, the sequence pairing *StXERICO1* with novel-miR1730-3p was integrated into the *pGreenII0800*-LUC (LUC) vector to obtain the *StXERICO1*-LUC recombinant plasmid. Subsequently, mutations were introduced to the target gene sequence sites while maintaining the amino acid sequence unchanged, forming multiple mismatch bases. This modified construct was fused with the LUC vector and named *mXERICO1*-LUC. A novel-miR1730-3p precursor sequence was cloned from the CIP706205 genome using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China). The novel-miR1730-3p precursor sequence was then constructed into the PEZR_(K)-LC vector and named LC-*Pre-miR1730-3p*. The constructed plasmid was transformed into *A. tumefaciens* (GV3101, pSoup-P19). Positive clones were selected and cultured overnight at 28 °C with shaking at 200 rpm. The bacteria were then suspended in MMA solution (10 mM MES, 10 mM $MgCl_2$, 0.2 mM acetosyringone, pH 5.8) until the OD_{600} concentration reached approximately 0.7–0.8 and were co-transformed into *N. benthamiana* leaves. Samples were taken from the *N. benthamiana* leaves infected with *A. tumefaciens*, and the activity of LUC (Firefly luciferases) and REN (Renilla luciferases) was determined using the Dual Luciferase Reporter Gene Assay Kit (Beyotime Biotechnology, Shanghai, China). Each experiment included three independent biological replicates.

2.9. Construction Virus-Induced Silencing Vector and Virus-Based MicroRNA Silencing

STTM_novel-miR1730-3p was amplified with three primers. After PCR amplification, the PCR product was gel-purified and ligated into the pTRV2 vector using the In-Fusion technique. This ligation was performed with the ClonExpress II One Step Cloning Kit (Vazyme, Nanjing, China).

TRV2-*GFP*, TRV2-*PDS*, TRV2-*STTM_novel-miR1730-3p*, and TRV1 vectors were transformed into *A. tumefaciens* (GV3101, pSoup-P19). Positive clones were selected and incubated overnight at 28 °C with shaking at 200 rpm. The bacteria were suspended in MMA solution (10 mM MES, 10 mM $MgCl_2$, 0.2 mM acetosyringone, pH 5.8) until the OD_{600} concentration reached approximately 0.7–0.8. Co-cultivation of TRV2-*GFP*, TRV2-*PDS*, TRV2-*STTM_novel-miR1730-3p*, and TRV1 was performed separately. The stem sections of *S. phureja* tissue culture plantlets (CIP 703541), cultured for 3 weeks, were soaked in the resuspension of the corresponding combination after the roots were trimmed with sterile scissors and then transplanted into the soil 15 min later. Ten stem sections per combination were planted in a temperature-controlled and moisture-controlled incubator. After 3 weeks, the newly sprouted leaves were harvested for RNA and miRNA extraction, which were then converted into cDNA for qRT-PCR to assess the silencing efficiency of silenced plants. Three biological replicates and three corresponding technical replicates were used for each qRT-PCR.

2.10. Yeast Two-Hybrid (Y2H) Assay

The protein translated by *StXERICO1* was submitted to the STRING online software (Version: 11.0) for predicting interacting proteins, selecting *Arabidopsis thaliana* as the reference organism. High-scoring interactions were identified with StUBC and StTLP proteins. To verify whether StXERICO1 interacts with StUBC and StTLP, we constructed the pGBKT7-*StXERICO1* decoy vector, as well as the StUBC-AD and StTLP-AD prey vectors. The pGBKT7-*StXERICO1* plasmid was transferred into Y2H Competent cells, while the StUBC-AD and StTLP-AD plasmids were transferred into Y187 Competent cells. The toxicity and self-activation activity of the pGBKT7-*StXERICO1* decoy vector were verified on SD/-Trp and SD/-Trp-His-Ade-X-α-gal solid yeast medium. Subsequently, pGBKT7-*StXERICO1*, StUBC-AD, and StTLP-AD were co-cultured to produce hybrid offspring. They were inoculated on SD/-Trp-Leu double-dropout solid medium and SD/-Trp-Leu-Ade-His-X-α-gal quadruple-dropout solid yeast medium, respectively, and their growth was observed at 29 °C.

2.11. Statistical Analysis

Statistical analyses were performed using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA). The data were subjected to one-way analysis of variance (ANOVA) and are presented as means ± standard deviation, based on a minimum of three replicates. Mean comparisons were conducted using Duncan's test, with statistical significance defined as a *p*-value less than 0.05.

3. Results

3.1. Characterization and Spatial-Temporal Expression Analysis of StXERICO1 in Potato Plants

After carefully analyzing transcriptome data of diploid potato plants during drought–rehydration cycles [22], we found that a potato *XERICO* gene was highly responsive to drought stress. Based on the publicly available potato genome models [26] and our transcriptome data, this gene was cloned and named *StXERICO1*.

StXERICO1 contained a complete Open Reading Frame (ORF) 459 bp encoding 152 amino acids, which includes an N-terminal transmembrane (TM) domain and a RING finger domain located at the C-terminus (Figure 1A). Blast analysis revealed that the protein encoded by the *StXERICO1* gene shares 100% amino acid sequence identity with KAH0706774.1 in the NCBI database. Physicochemical properties and phosphorylation site analysis showed that the relative molecular weight of the StXERICO1 protein is 17.232 kDa, with a theoretical isoelectric pI of 5.03. The protein contains eleven Ser sites, three Thr sites, and one Tyr site, which may significantly impact its function. Phylogenetic analysis of the eight XERICO homologs demonstrated that monocotyledonous and dicotyledonous plants cluster into distinct branches, reflecting evolutionary conservation and clear divergence between these groups (Figure 1B). StXERICO1 is highly homologous within the Solanaceae family, showing 95.39% similarity to the XERICO protein in pepper. In contrast, the similarity between the potato XERICO protein and maize XERICO protein is only 34.60% (Figure 1C).

The *StXERICO1* gene exhibited varied expression across different organs of the potato plant, with higher expression levels observed in mature whole fruits, sepals, carpels, and stolons and the lowest levels in leaves (Figure 2A). *StXERICO1* expression was strongly induced by abiotic stresses and hormone treatments (Figure 2B). Specifically, after 24 h of mannitol treatment, *StXERICO1* expression increased by 30.88% compared to the control. In contrast, after 24 h at 35 °C, expression decreased to 1.46 times that of the control. Following 24 h of treatment with 50 μM GA3 and 50 μM ABA, *StXERICO1* expression increased by 0.46 times and 1.08 times, respectively (Figure 2B).

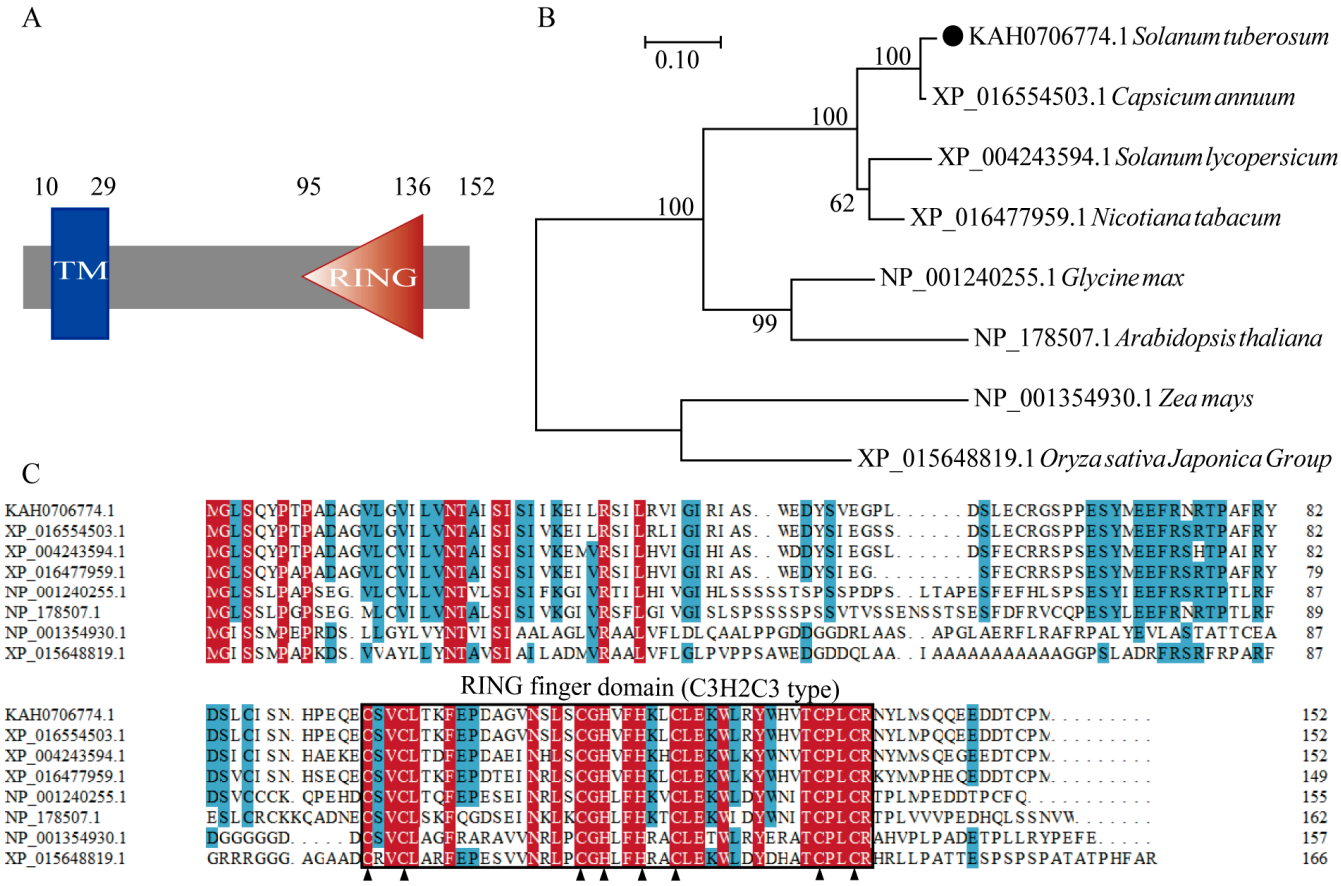

Figure 1. StXERICO1, a RING-H2 type E3 ubiquitin ligase, from *Solanum tuberosum* L. (**A**) Structure of the StXERICO1 protein showing the transmembrane domain (TM) and RING domain. (**B**) Phylogenetic tree of StXERICO1 from different plant species. The unrooted neighbor-joining phylogenetic tree based on StXERICO1 homologs was created using MEGA7.0. (**C**) The amino acid sequences of StXERICO1 and different plant species were aligned using ClustalW. The RING finger domain is highlighted by a black box, with the positions of cysteine (C) and histidine (H) indicated by black arrowheads. Identical amino acids are represented in red, while similar amino acids are shown in blue.

Notably, *StXERICO1* expression was highly responsive to drought stress, as demonstrated by our RNA-seq and qRT-PCR results. As shown in Figure 2C, *StXERICO1* expression exhibited rhythmic changes during cycles of drought, hydration, and re-drought. According to the qRT-PCR results, mild drought and re-drought induced significant increases in *StXERICO1* expression levels, with a 3.6-fold and 2.9-fold increase, respectively, compared to the corresponding control and the rehydration treatment. In contrast, rehydration after drought treatment significantly decreased the expression level, and severe drought and re-drought also led to a slight decline in *StXERICO1* expression. This suggests that *StXERICO1* can rapidly respond to drought stress. The RNA-seq and qRT-PCR results exhibited a similar trend, with a correlation coefficient of 0.821 (Figure 2C).

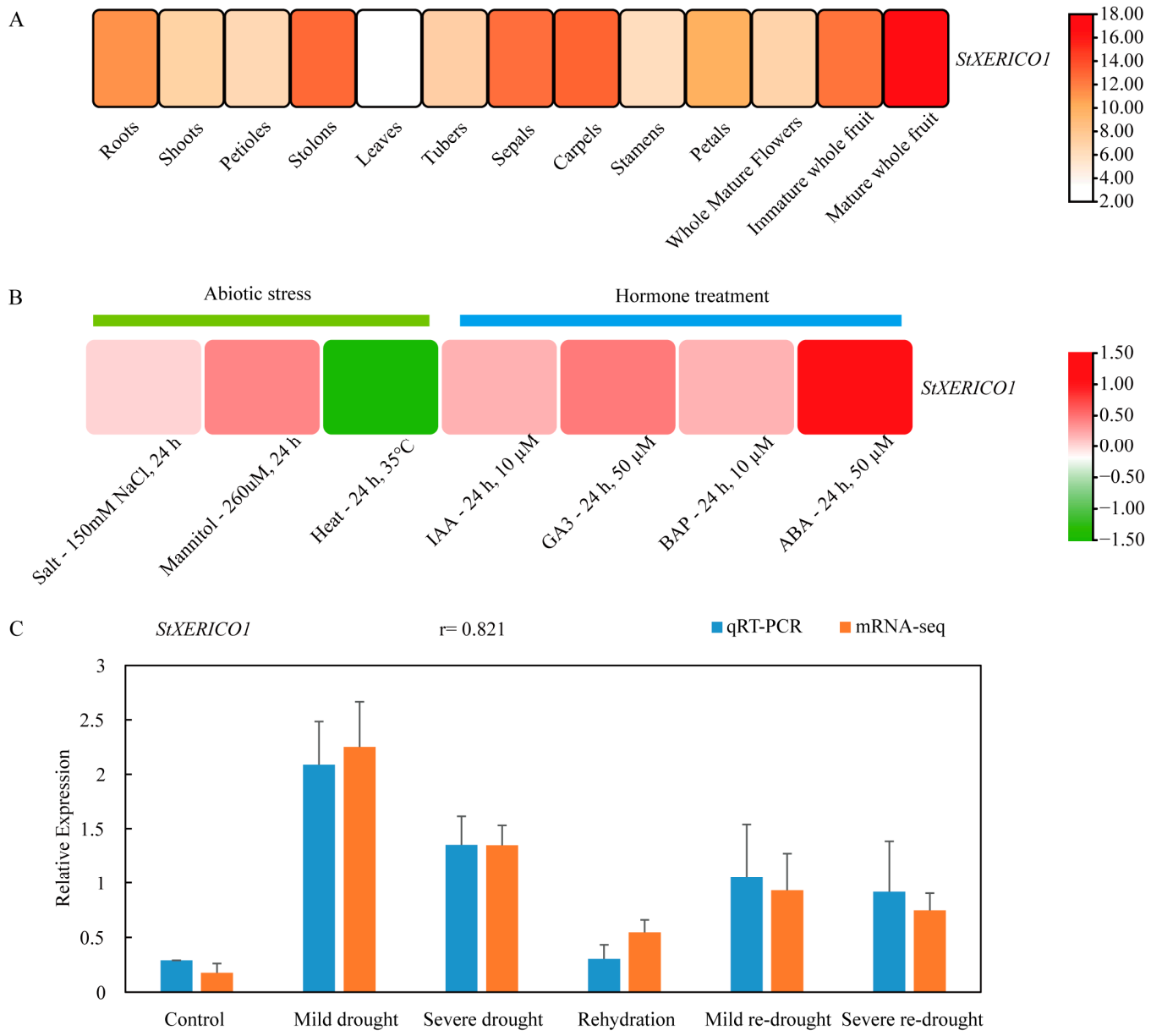

Figure 2. Expression patterns of *StXERICO1* in various organs of potato plants and its response to different treatments. (**A**) *StXERICO1* expression patterns across various organs of the potato plant. (**B**) Differential expression of *StXERICO1* in response to different treatments in potato plants. (**C**) Expression analysis of *StXERICO1* by qRT-PCR (blue) and RNA-seq (orange). The Pearson's correlation coefficient (r) is indicated in the upper middle corner. All expression values were normalized by dividing the means of the six treatments.

3.2. Effects of Overexpression and Knockout of StXERICO1 on Growth, Drought Resistance, and ABA Content in Potato Plants

To elucidate the function of the *StXERICO1* gene, overexpression and knockout experiments were conducted in diploid potato. In the overexpression experiment, a total of 138 regenerated plantlets were obtained, from which 28 diploid plantlets exhibiting overexpression were identified through flow cytometry analysis and PCR verification (Figure 3A). Subsequently, the *StXERICO1* overexpression levels in 10 of these diploid lines were analyzed by qRT-PCR. As shown in Figure 3B, *StXERICO1* expression levels in the 10 transgenic lines were overall significantly higher than those in the WT (wild type) and empty vector controls, indicating that overexpression of *StXERICO1* greatly enhanced its expression level in transgenic potato plants. From these lines, we selected three that represented relatively high,

medium, and low expression levels, labeled 5#, 34#, and 87#, respectively. The *StXERICO1* expression levels in these lines were 90-, 40-, and 20-fold higher than those in the WT and empty vector controls, respectively, for further experimentation.

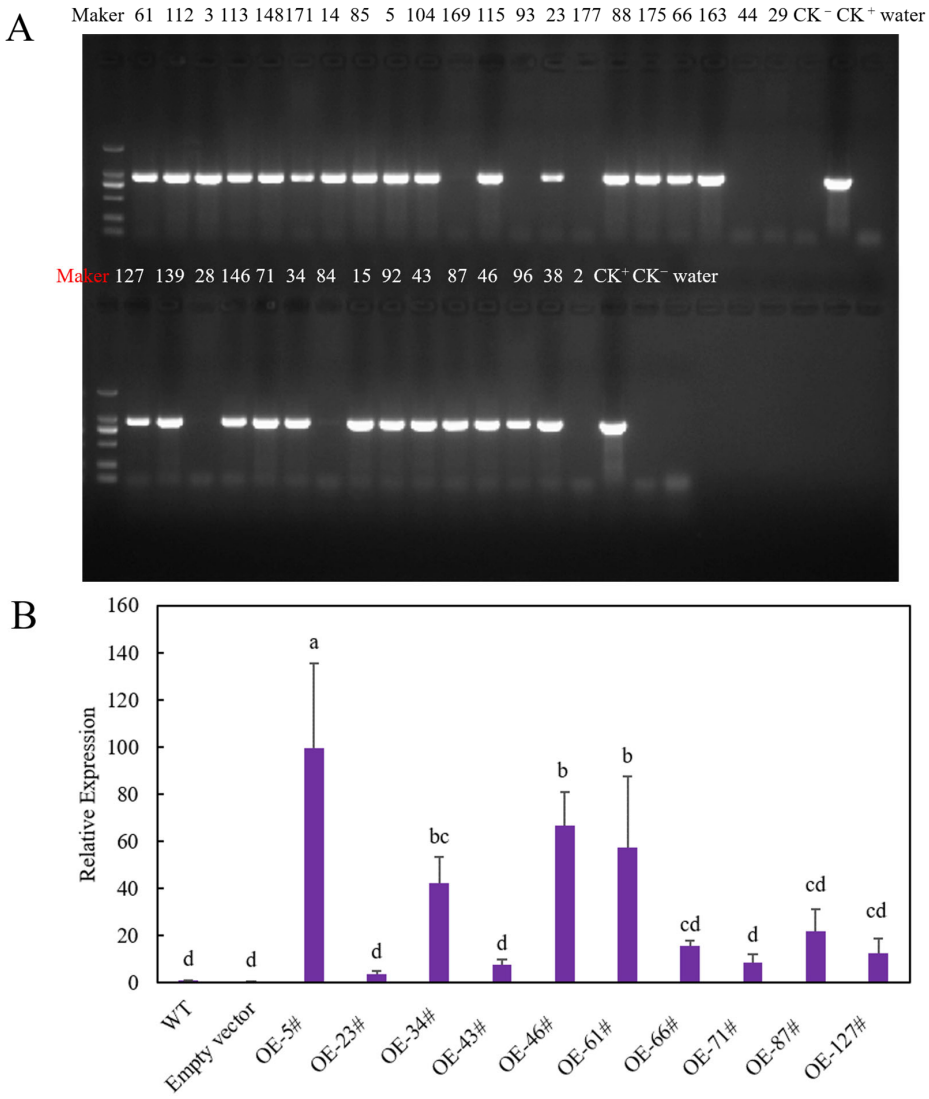

Figure 3. PCR verification (**A**) and qRT-PCR analyses (**B**) of *StXERICO1* between WT, empty vector, and transgenic potato lines. Each bar indicates the mean ± SD from three biological replicates. Different letters indicate statistically significant differences ($p \leq 0.05$). CK^+: template is positive plasmid, CK^-: template is WT, Water: template is water. The numbers represent different transgenic potato lines.

To further explore the function of *StXERICO1*, we attempted to produce null mutants by targeting an editing site on the exon of *StXERICO1* using CRISPR-Cas9-mediated genome editing. A single-target knockout vector was constructed as shown in Figure 4A, using our previously described method [24]. After co-culture with *Agrobacterium tumefaciens* containing the knockout vector, 22 regenerated plantlets were obtained. Flow cytometry analysis revealed that only three diploid genome-editing lines, KO-48#, KO-29#, and KO-6#, were successfully created. Sequencing of the target gene fragment indicated that one homozygous diploid mutant line, KO-48#, was finally obtained with a 1-nucleotide insertion (Figure 4B), which was used for subsequent experiments.

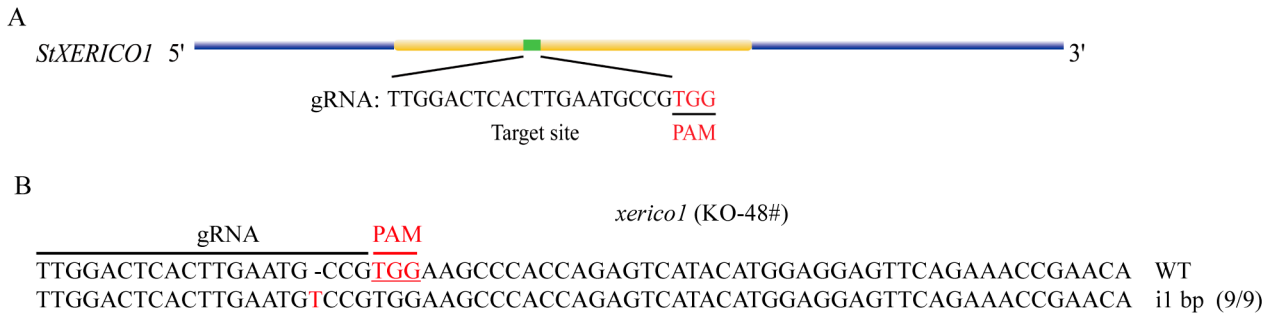

Figure 4. The schematic diagram of *StXERICO1* mutation patterns. (**A**) The schematic diagram of *StXERICO1* with target sites. gRNA sequences and PAM are marked. (**B**) The mutation pattern of *StXERICO1* in transgenic line. i1 bp means insertion of 1 bp. The insertion is shown in red capital letter T.

Phenotypic observations of these transgenically modified potato plants showed that *StXERICO1* overexpression inhibited shoot and root growth to some extent, which generally correlated with the overexpression levels of *StXERICO1*. For example, lines 5# and 34#, which exhibited higher overexpression levels (Figure 3), also showed greater growth inhibition in shoots and roots. Conversely, line 87#, which had lower overexpression levels, demonstrated no significant growth inhibition (Figure 5). On the other hand, the mutant line, KO-48#, which contains a 1-nucleotide insertion (Figure 4), promoted shoot and root growth in the mutant plantlets to some extent compared to the WT control (Figure 5).

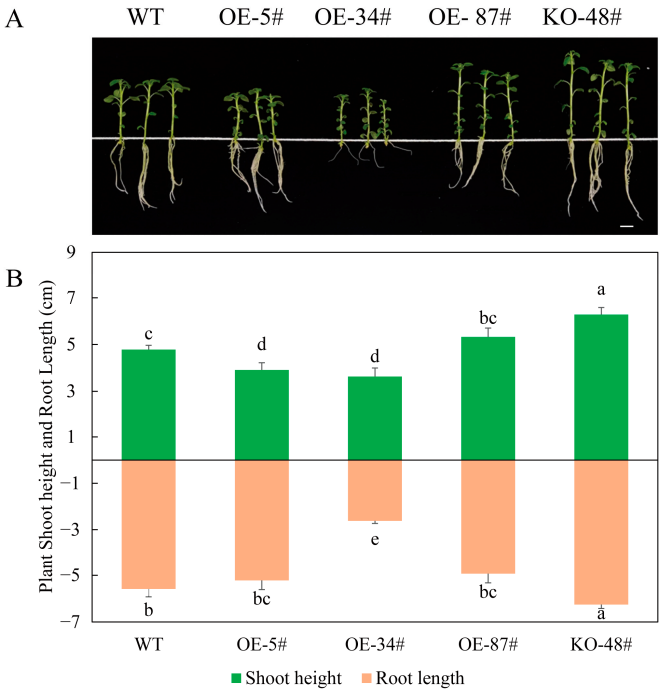

Figure 5. Effects of overexpression and knockout of *StXERICO1* on shoot and root growth in potato plantlets. (**A**) Phenotypic observation of transgenic lines and wild-type potato plantlets. (**B**) Measurement of shoot height and root length in potato plantlets. Representative pictures were taken after 2 weeks of tissue culture. Scale bar, 1 cm. Different letters indicate statistically significant differences ($p \leq 0.05$).

To test the responses of these transgenically modified potato lines to ABA sensitivity, terminal shoots from potato plants of the same age were cut to equal lengths and transplanted into MS medium without ABA and with 15 or 20 μM ABA, respectively. After two weeks of culture, the growth phenotype was observed, and the length of regenerated roots was

measured. As shown in Figure 6, after two weeks of treatment with 15 µM ABA, both the transgenic lines and WT exhibited decreased root growth; notably, the OE-5# line showed almost no rooting. Following two weeks of 20 µM ABA treatment, it was evident that the WT could still root to some extent, while the three overexpression lines showed almost no root development (Figure 6, the WT control shows a root length of 0.99 cm). On the other hand, the rooting performance of the knockout line KO-48# was similar to those of the wild type (WT) when grown in an MS medium supplemented with 15 or 20 µM ABA (Figure 6).

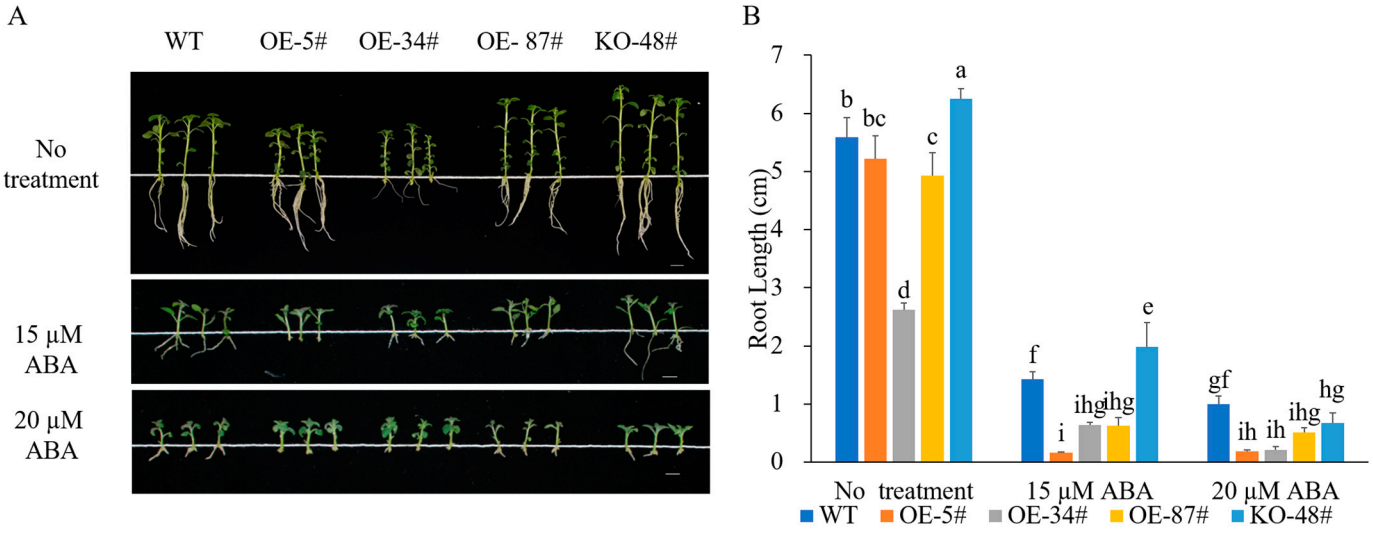

Figure 6. Overexpression of *StXERICO1* confers hypersensitivity to ABA treatment in transgenic lines of potato. (**A**) Effects of ABA treatment on the root growth of WT and transgenic lines. Representative pictures were taken two weeks after terminal shoots were grown in MS medium supplemented with 15 µM ABA, and 20 µM ABA, respectively. Scale bar, 1 cm. (**B**) Measurement of root length in potato plantlets. Each bar indicates the mean ± SD from three biological replicates. Different letters indicate statistically significant differences ($p \leq 0.05$).

To evaluate the drought resistance of these genetically modified potato lines, the plants cultured on solid MS medium were transplanted in nutrient soil and grown in an incubator for 34 days with regular watering. Watering was then discontinued to observe the drought resistance phenotypes. As shown in Figure 7A, after 17 days of drought treatment, the plants of both WT and the knockout line KO-48# exhibited severe wilt, and some even died. In contrast, the three *StXERICO1* overexpression lines showed less wilt and demonstrated significantly better drought resistance. After uniformly rehydrating the drought-treated plants, the surviving plants returned to normal growth to some extent. However, the lower leaves of both the wild type and the knockout line could not fully recover, and some exhibited necrosis. The results clearly showed that overexpression of *StXERICO1* in potato plants significantly enhanced their drought resistance. In addition, the knockout line KO-48#, with a 1-nucleotide insertion, did not significantly alter its drought resistance compared to the WT control in this experiment (Figure 7A).

Given that *StXERICO1* overexpression can promote drought tolerance by regulating abscisic acid (ABA) levels in plants [27], we measured the endogenous ABA content of the overexpressed and wild-type lines. The results indicated that, following drought treatment, the endogenous ABA contents in the three overexpression lines were significantly higher than in the wild-type line (Figure 7B). In fact, ABA content was positively correlated with the levels of *StXERICO1* overexpression in the selected lines 5#, 34#, and 87#, which represented relatively high, medium, and low overexpression levels, respectively (Figures 3 and 7B).

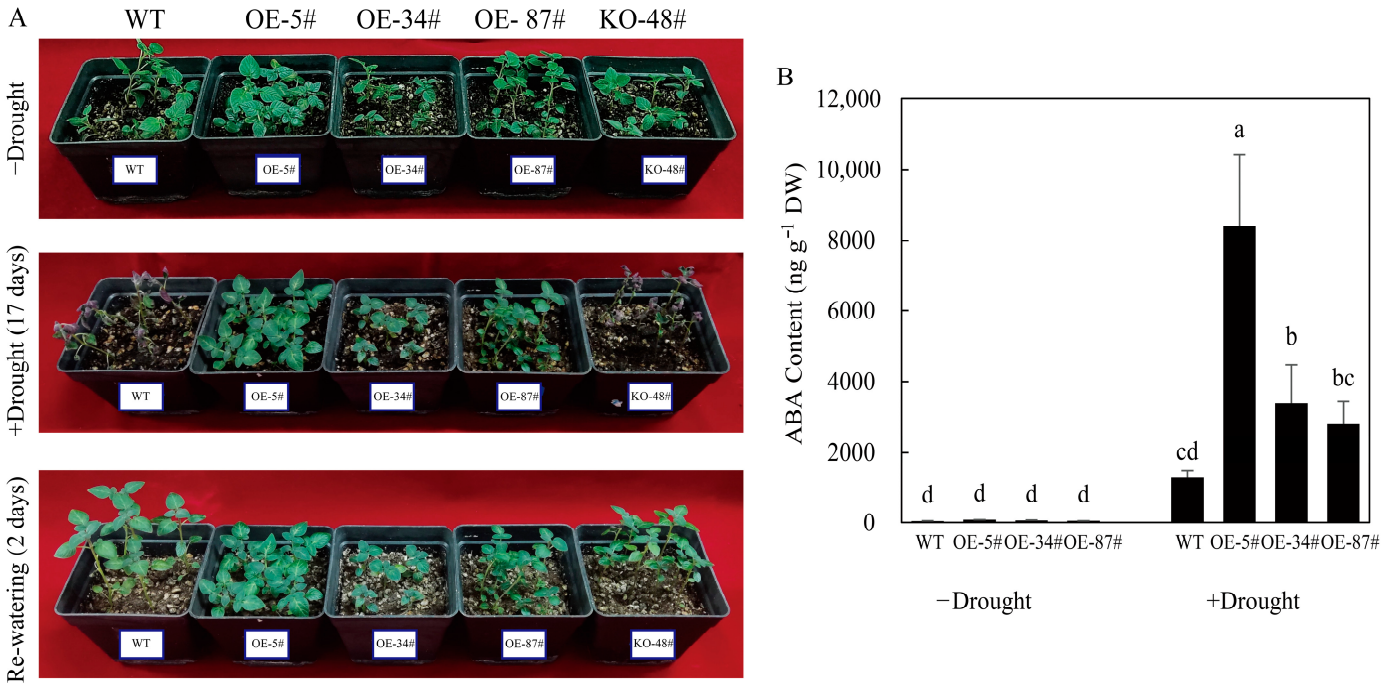

Figure 7. Overexpression of *StXERICO1* enhances drought resistance and endogenous ABA level in transgenic potato plants. (**A**) Wild-type and transgenic potato lines planted for 34 days in nutrient soil were subjected to drought stress for 17 days followed by rehydration. Representative pictures were taken at 17 days without watering, as well as 2 days after re-watering. (**B**) Accumulation of ABA between WT and three transgenic lines. Each bar indicates the mean ± SD from three biological replicates. Different letters indicate statistically significant differences ($p \leq 0.05$).

3.3. Effects of Overexpression of StXERICO1 on Drought Resistance and ABA Content in Tobacco Plants

To further understand the function of the potato *StXERICO1* gene in cross-species, a *StXERICO1* overexpression experiment was conducted in tobacco by leaf disk genetic transformation mediated by *Agrobacterium*. A total of four regenerated plantlets and T0 seeds were screened and obtained. Subsequently, we selected three *StXERICO1* overexpression lines labeled X1, X2, and X3, respectively. The *StXERICO1* expression levels in these lines were 569-, 169-, and 261-fold higher than those in the WT controls, respectively, for further experimentation (Figure 8C). Their growth phenotypes are shown in Figure 8B.

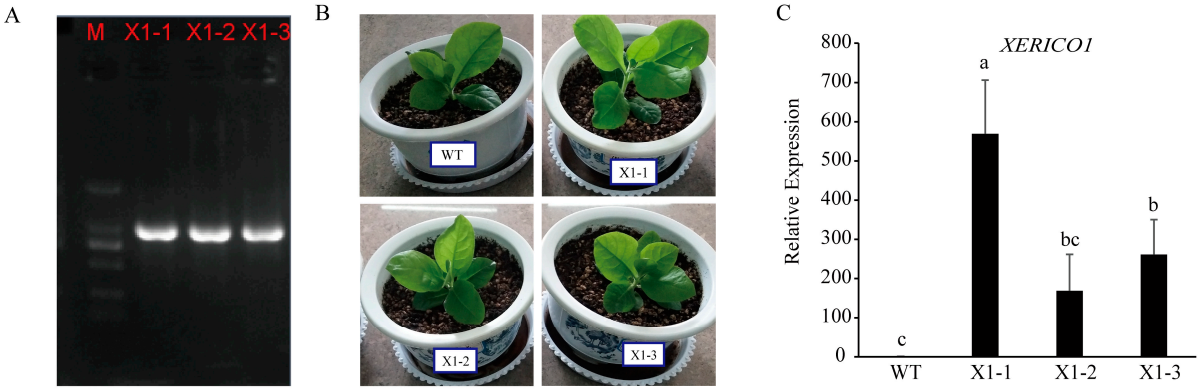

Figure 8. PCR verification (**A**), growth phenotype observation (**B**) and qRT-PCR analysis of *XERICO1* mRNA levels (**C**) in WT and three independent T1 transgenic tobacco lines. Each bar indicates the mean ± SD from three biological replicates. Different letters indicate statistically significant differences ($p \leq 0.05$).

To further evaluate the effect of *StXERICO1* on drought resistance in transgenic tobacco lines, the wild type (WT) and T1 tobacco seeds overexpressing *StXERICO1* were sown in soil and grown in an incubator for one month with regular watering. Watering was then discontinued to observe the drought resistance phenotypes. As shown in Figure 9, after 16 days of drought treatment, the plants of both WT and transgenic lines exhibited severe leaf wilting. After uniformly rehydrating the drought-treated plants, most transgenic lines showed leaf recovery, whereas the wild-type tobacco did not recover significantly. Upon seven days of rehydration, the leaf growth of transgenic lines was notably superior to that observed after 2 days of rehydration, while the leaves of wild-type tobacco still exhibited limited recovery. The data indicate that the overexpression of the *StXERICO1* gene confers higher drought resistance compared to WT (Figure 9).

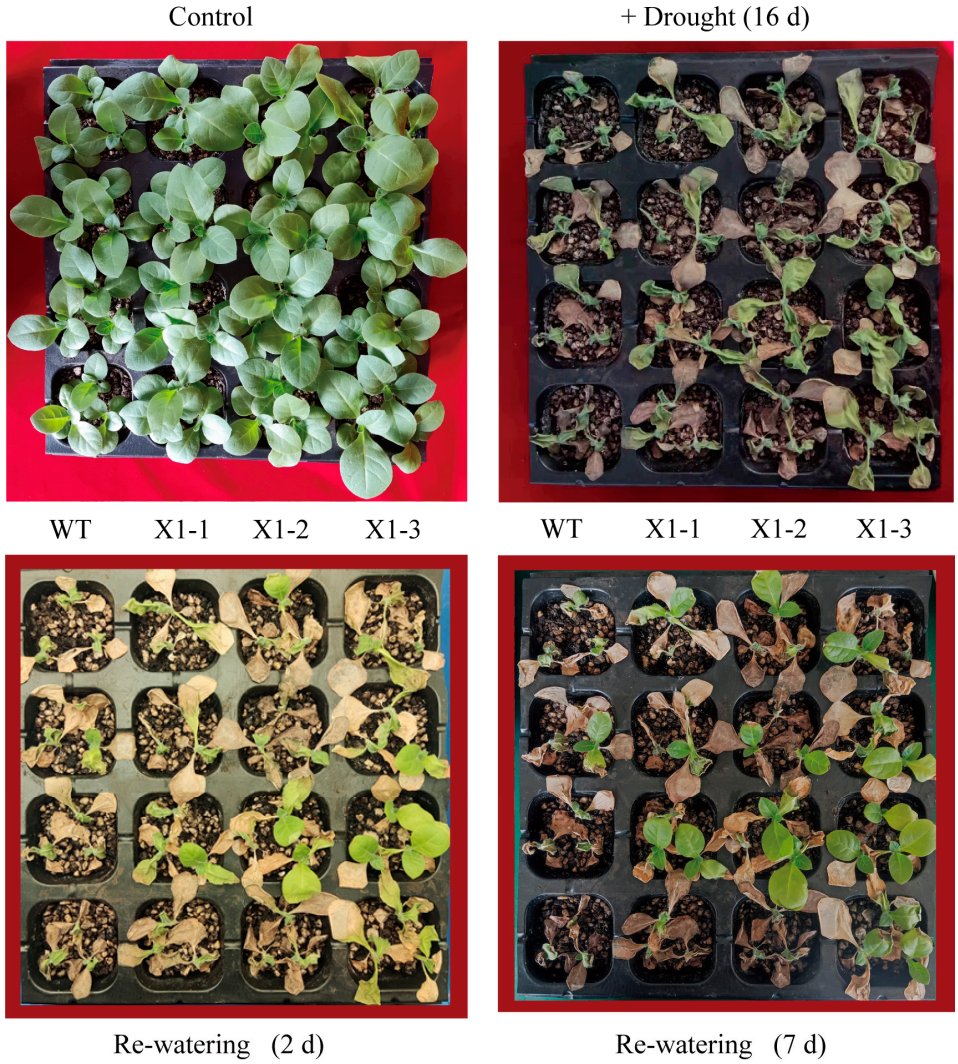

Figure 9. Phenotypic analysis of drought resistance in tobacco plants overexpressing *StXERICO1*. Wild-type and transgenic tobacco plants were subjected to drought stress for 16 days, followed by a 7-day rehydration period. Representative photographs were taken at the start of the drought stress (day 0, control), at the end of the stress period (day 16), and then 2 and 7 days after re-watering.

In this study, three transgenic tobacco lines with high expression of *StXERICO1* were identified (Figure 8C). *NCED*, a key gene in the ABA synthesis pathway, and *CYP707A*, a key gene in ABA metabolism, were analyzed using qRT-PCR. The results showed that the expressions of *NtNCED* and *NtCYP707A* increased in these three transgenic lines, overexpressing *StXERICO1* to some extent (Figure 10A,B). In addition, the content of endogenous ABA in the transgenic lines X1 and X2 was also significantly increased (Figure 10C), indi-

cating that the overexpression of potato *StXERICO1* gene increased the endogenous ABA content in the transgenic tobacco lines.

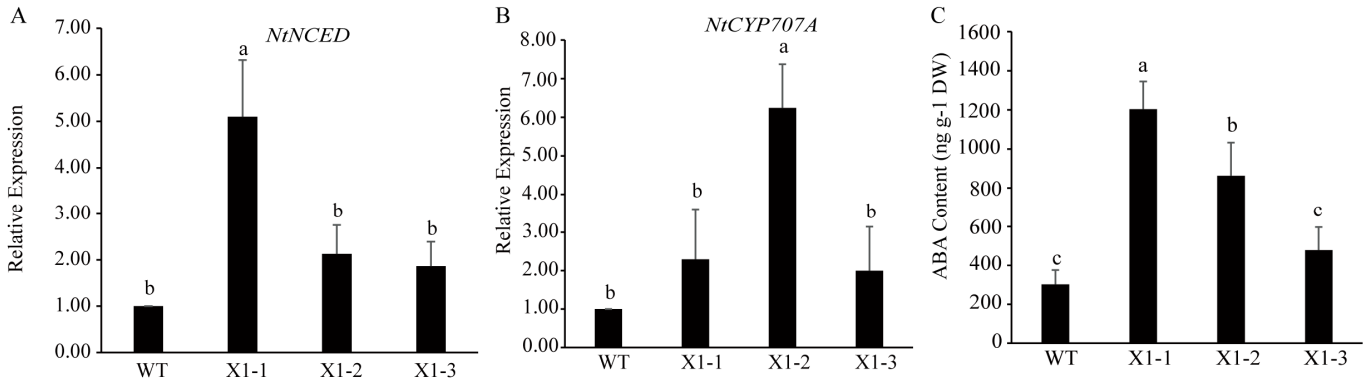

Figure 10. qRT-PCR analysis of *NtNCED* (**A**), and *NtCYP707A* (**B**) expression levels, along with the determination of endogenous ABA content (**C**) in the WT and three transgenic tobacco lines overexpressing *StXERICO1*. Each bar represents the mean ± SD from three biological replicates. Different letters indicate statistically significant differences ($p \leq 0.05$).

3.4. Analysis of StXERICO1 Interactions with miRNAs and Confirmation of StXERICO1 as a Target of the Novel-miR1730-3p

Using the bioinformatics website psRNATarget and integrating our whole transcriptome sequencing data (including miRNAome data), we predicted that a total of 75 miRNAs could potentially target *StXERICO1*, as illustrated in Table S2.

To further verify the direct interaction between *StXERICO1* and a specific miRNA, we selected novel-miR1730-3p from our miRNAome data, which is highly responsive to drought and abundant, to study their interaction using 5′ RLM-RACE. The results indicated that the transcript of *StXERICO1* was cleaved between the 7th and 8th bases of the 5′ UTR by novel-miR1730-3p, and this cleavage occurred at this location in nine out of the ten selected clones (Figure 11).

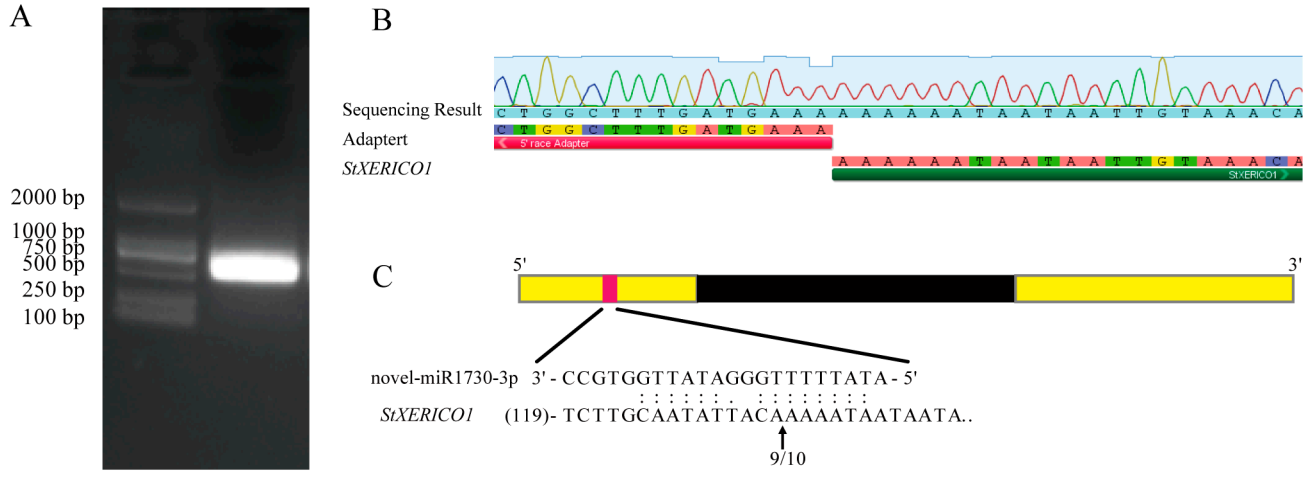

Figure 11. Validation of *StXERICO1* as a target for novel-miR1730-3p using 5′-RLM-RACE. (**A**) Agarose gel image of 5′ RACE product. (**B**) Sequencing result of the PCR product. (**C**) The targeted *StXERICO1* section and the miRNA sequence. The gene's coding region is represented in black, while the UTR region is in yellow. The putative cleavage site is indicated by a dark pink symbol. The vertical arrowheads denote the 5′ ends of the cleaved product, accompanied by the number of clones analyzed for *StXERICO1*.

To further assess the direct interaction between *StXERICO1* and novel-miR1730-3p, two effector vectors and two reporter vectors were constructed (Figure 12). The inhibitory effect of the novel-miR1730-3p on *StXERICO1* expression was validated using a dual luciferase assay. The results from the dual luciferase assay showed that co-expression of *StXERICO1* with novel-miR1730-3p significantly decreased the relative LUC/REN value. However, this inhibitory effect was diminished when *mStXERICO1* (mutant *StXERICO1)* was co-expressed with novel-miR1730-3p (Figure 12C), indicating that novel-miR1730-3p decreased the expression level of *StXERICO1*.

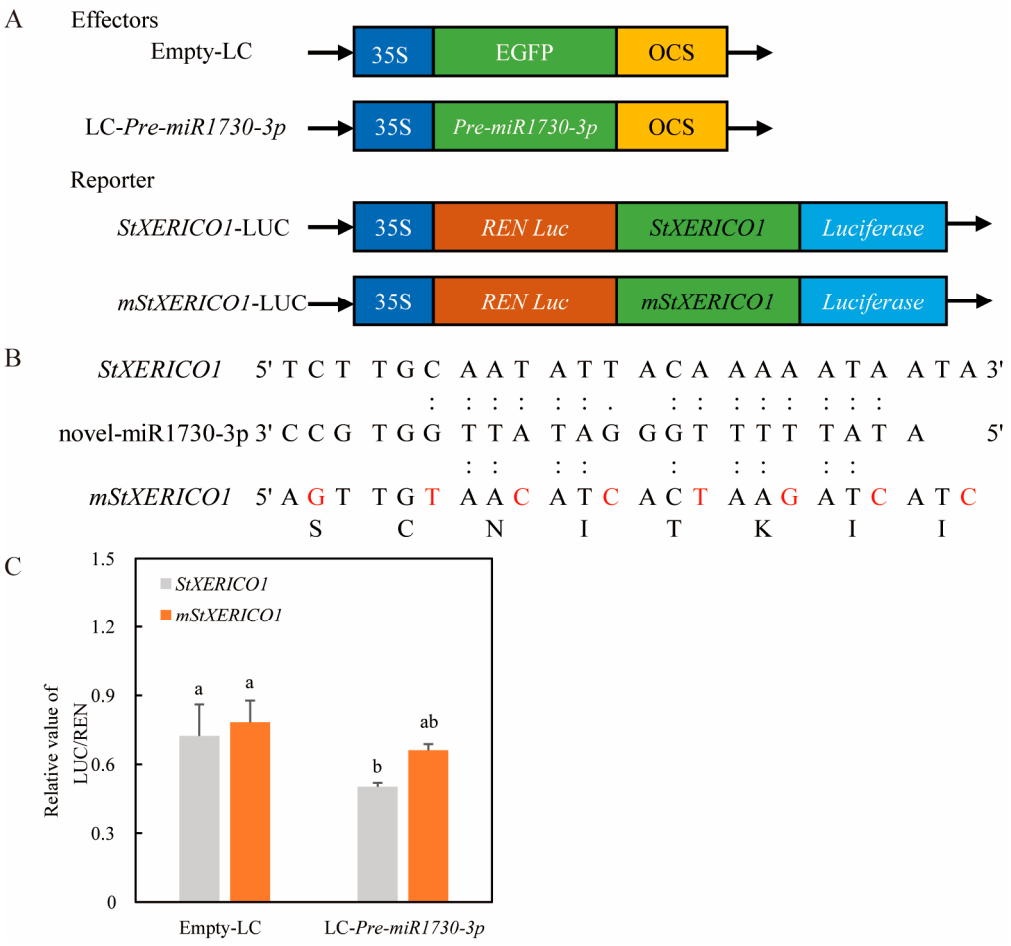

Figure 12. Novel-miR1730-3p inhibited the accumulation of *StXERICO1* transcript. (**A**) Schematic representation of gene constructs: Effector vectors are Empty-LC or LC-*Pre-m1730-3p*, and reporter vectors are *StXERICO1*-LUC or *mStXERICO1*-LUC. (**B**) Schematic representation of *StXERICO1* and *mStXERICO1*. Although the amino acid sequence remains unchanged; mutations were introduced into the novel-miR1730-3p-*StXERICO1* matching sequence, resulting in *mStXERICO1*. Two points represent perfectly matched base pairs, while red indicates mutant bases. (**C**) Dual luciferase reporter assay. Each bar indicates the mean ± SD of three biological replicates. Different letters indicate statistically significant differences ($p \leq 0.05$).

To confirm that novel-miR1730-3p indeed negatively regulated the expression of *StXERICO1* in potato, a novel-miR1730-3p gene silencing vector was constructed using virus-based microRNA silencing technology (Figure 13A,B). As shown in Figure 13C, the novel-miR1730-3p gene-silenced potato plants (TRV_STTM1730) were successfully obtained. Compared with the TRV control, *StXERICO1* expression was significantly up-regulated in the novel-miR1730-3p-silenced plants (TRV_STTM1730) when novel-miR1730-3p expression was inhibited in the novel-miR1730-3p-silenced plants (Figure 13D). These experiments conclu-

sively demonstrated that novel-miR1730-3p can target and cleave *StXERICO1* transcripts, thereby inhibiting their accumulation in potato plants.

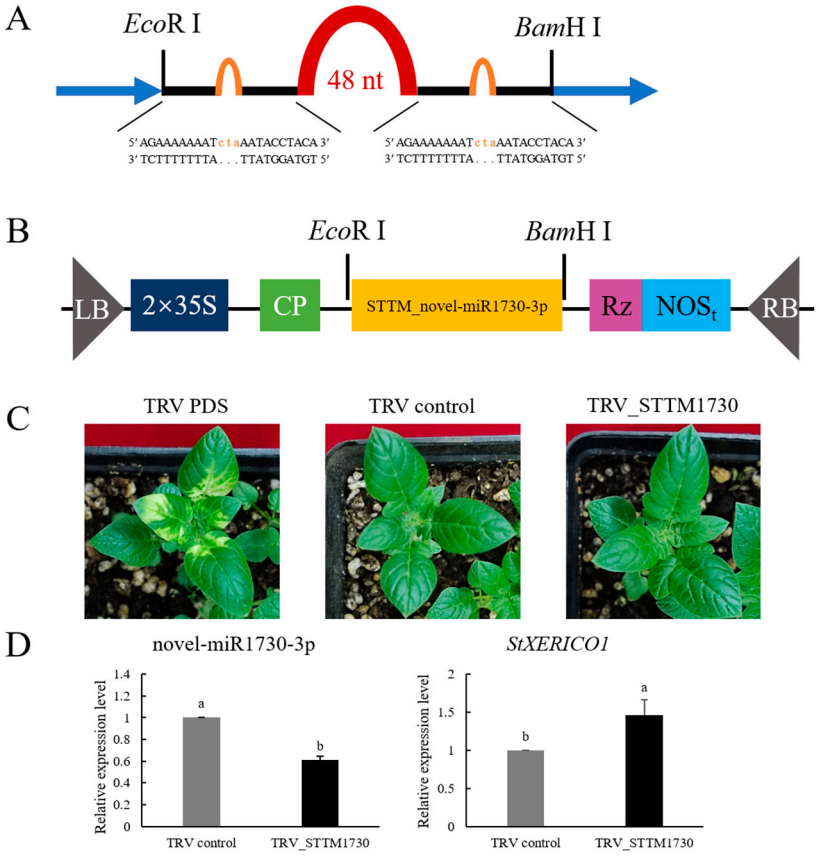

Figure 13. Virus-based silencing of novel-miR1730-3p using the STTM approach in potato. (**A**) Diagrammatic representation of novel-miR1730-3p structure. (**B**) Diagrammatic representation of STTM vector. (**C**) Diagrammatic representation of sampling timeline. The TRV PDS plants served as a positive control, which was sampled when leaf lesions appeared. The TRV control plants served as a negative control. The TRV_STTM1730 plants were novel-miR1730-3*p*-silenced plants. Representative images were taken 20 days after planting. (**D**) Detection of silencing efficiency of novel-miR1730-3p and its target *StXERICO1*. Each bar indicates the mean ± SD of three biological replicates. Different letters indicate statistically significant differences ($p \leq 0.05$).

3.5. Analysis of the Interaction between StXERICO1 Protein and StUBC and StTLP Proteins

Using the STRING Online software (version: 11.0), which is designed to predict protein interactions, we screened two high-scoring interactions involving StUBC and StTLP proteins. StUBC (accession number Soltu.DM.03G022950.1) is annotated as an E2 ubiquitin ligase, while StTLP (accession number Soltu.DM.09G022900.1) is characterized as a Tubby-like protein [26]. The results from the yeast two-hybrid assay showed that the pGBKT7-*StXERICO1* grew colonies on SD/-Trp media, indicating that the pGBKT7-*StXERICO1* prey vector did not exhibit toxicity (Figure 14A). The pGBKT7-*StXERICO1* construct did not yield colonies on SD/-Trp/-His/-Ade triple-deficient media with X-α-gal, indicating that pGBKT7-*StXERICO1* did not exhibit self-activating activity (Figure 14B). The positive control (pGADT7-T+pGBKT7-53) and experimental groups (pGBKT7-*StXERICO1*+pGADT7-*StUBC*, pGBKT7-*StXERICO1*+ pGADT7-*StTLP*) grew colonies on both two- and four-deficient media with X-α-gal, and the colonies turned blue on four-deficient media coated with X-α-gal. In contrast, the negative control (pGADT7-T+pGBKT7-lam) group formed colonies on two-deficient media but did not grow colonies on all four-deficient media

(Figure 14C,D), suggesting an interaction between the StXERICO1-StUBC and StXERICO1-StTLP modules.

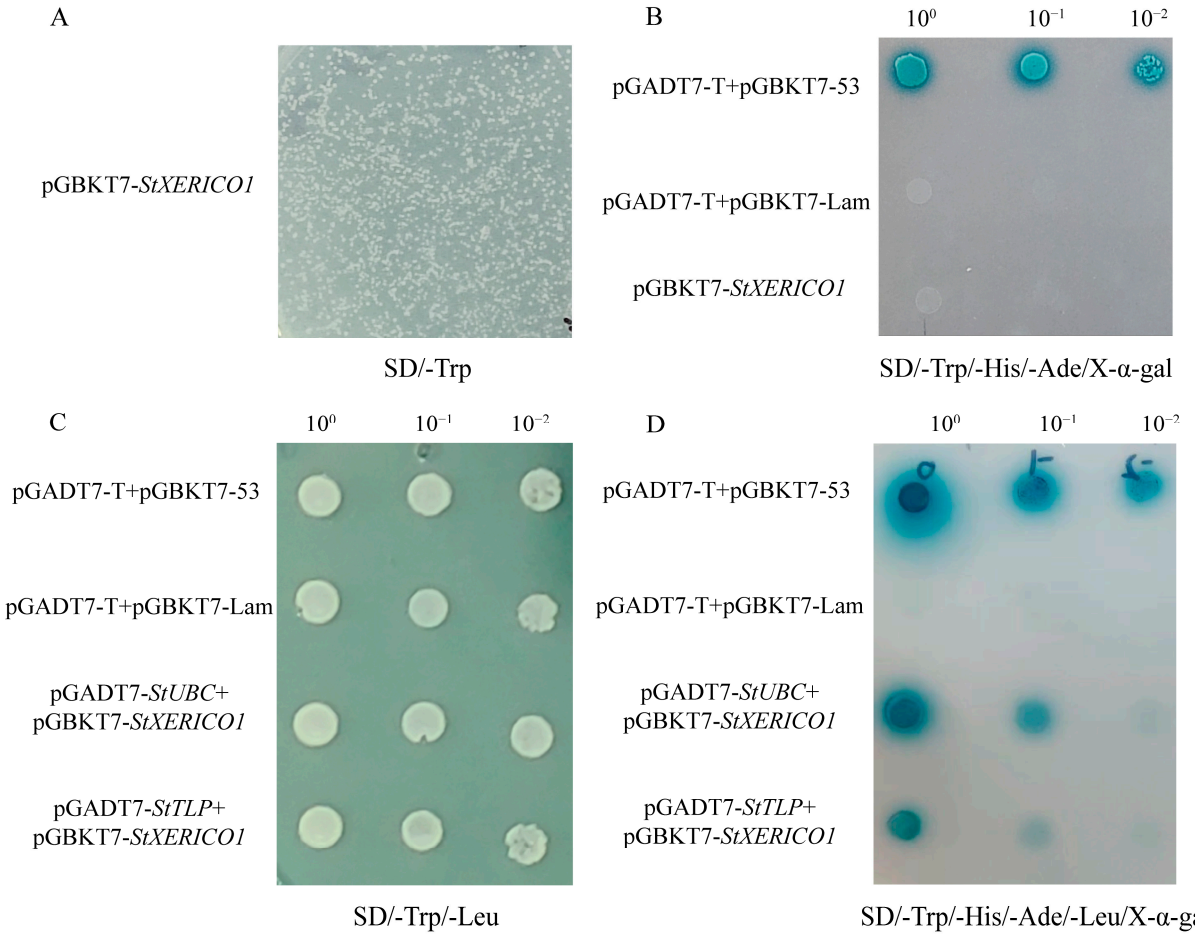

Figure 14. StXERICO1 interacts in vivo with StUBC and StTLP in yeast. (**A**) Toxicity validation of the pGBKT7-*StXERICO1* prey vector. (**B**) Validation of self-activation for the pGBKT7-*StXERICO1* prey vector. (**C**,**D**) Validation of StXERICO1-StBUC and StXERICO1-StTLP interaction. Positive control: pGADT7-T+pGBKT7-53, negative control: pGADT7-T+pGBKT7-Lam.

4. Discussion

As a drought-sensitive crop, drought resistance in potato is a crucial trait that enables them to survive and thrive under conditions of water scarcity. Various genetic and molecular pathways have been identified to contribute to this complex trait [27–29]. *XERICO*, first identified in *Arabidopsis thaliana*, encodes an E3 ubiquitin ligase and has emerged as a significant player in the regulation of drought tolerance, which plays a pivotal role in abscisic acid (ABA) biosynthesis and signaling [18–21]. ABA is a key phytohormone involved in the regulation of plant responses to abiotic stress, particularly drought [30]. Overexpression of *XERICO* has been shown to confer drought tolerance and increase ABA accumulation in *Arabidopsis* and other plant species [19,20,25]. XERICO-mediated accumulation of ABA leads to the activation of ABA-responsive transcription factors, which in turn induce the expression of a variety of stress-responsive genes. These genes encode proteins involved in osmoprotection, detoxification, and cellular protection, which are crucial for plant survival under drought conditions [18–21,25].

In this study, we cloned the *StXERICO1* gene from potato, which has an open reading frame of 459 bp encoding 152 amino acids. The protein it encodes features a structure composed of α-helices and random curls, and it shows sequence similarity to XERICO proteins from other species, all of which contain conserved TM and RING domains (Figure 1A).

Phylogenetic analysis revealed that StXERICO1 shares a high degree of similarity with plants from the Solanaceae family as well as monocotyledonous and dicotyledonous plants, but they are grouped into separate branches, demonstrating evolutionary conservation and distinct divergence between the different plant species (Figure 1B,C). This is the first reported sequence of this gene in the potato CIP706205 (*Solanum ajanhuiri* Juz. & Bukasov, Jancko Sisu Yari).

Genes induced by exogenous ABA treatment are recognized for their involvement in ABA-dependent stress response pathways [31]. *StXERICO1* was upregulated by ABA treatment (Figure 2B), and its expression responded to the drought-rewatering cycle, suggesting its role as a drought-responsive gene. This finding has not been previously reported in earlier studies, either through analysis or experimentation. Specifically, it exhibited an increased expression during drought and decreased expression during rehydration (Figure 2C), a pattern not previously reported. Drought-responsive genes help plants better adapt to drought conditions and enhance their drought resistance [25]. Therefore, *StXERICO1* may play a role in improving drought resistance in potato plants.

As shown in Figures 7A and 9, overexpression of the *StXERICO1* gene clearly alleviated drought-induced wilting and plants' injury, allowing better recovery upon re-watering. This indicates that overexpression of the *StXERICO1* gene indeed enhanced drought resistance in transgenic potato and tobacco plants (Figures 7A and 9). Furthermore, the transgenic potato and tobacco plants overexpressed *StXERICO1* demonstrated significantly higher ABA content than their corresponding controls under both watering or drought stress conditions (Figures 7B and 10C), indicating overexpression of the *StXERICO1* gene indeed induced ABA accumulation. In addition, the measurement of shoot and root length in transgenic potato lines treated with different concentrations of ABA revealed that overexpressed *StXERICO1* potato lines exhibited greater sensitivity to ABA treatment. These results suggest a significant regulatory role for *StXERICO1* in modulating plant responses to ABA. In our present experiment, however, knockout of *StXERICO1* with a 1-nucleotide insertion mutation via CRISPR-Cas9-mediated genome editing (line KO-48#) did not result in a noticeable decline in drought resistance or reduced sensitivity to ABA treatment in these transgenic potato plants (Figures 7A and 6), as predicted in our previous experimental design. This may be due to the presence of one more *XERICO* gene in potato, as occurred in maize [19], where gene redundancy provides a protective mechanism against the malfunction of one *XERICO* gene. The detailed mechanism awaits further investigation.

NCED and *CYP707A* are the key genes in the ABA synthesis pathway [32]. Our results showed that overexpression of *StXERICO1* significantly enhanced the expression levels of *NtNCED* and *NtCYP707A* in transgenic tobacco plants (Figure 10A,B), which may explain the observed increase in ABA content in the transgenic potato and tobacco plants. These findings further indicate that overexpression of the *StXERICO1* gene enhances drought resistance in potato and tobacco plants by regulating the expression of ABA biosynthetic genes and increasing endogenous ABA content.

MicroRNAs, typically ranging from 20 to 24 nucleotides in length, carry out their functions primarily through complementary binding to specific target sites. This interaction can result in either the degradation of the target mRNA or the repression of translation [33]. While it is well known that miRNAs interact with numerous genes involved in plant stress response and adaptation, little is known about the interaction between the *XERICO* gene and miRNAs. The virus-based microRNA silencing (VBMS) system has been developed and widely applied for various plant species [34,35]. Unlike other studies [36–38], we developed a protocol employing tobacco rattle virus (TRV)-based VBMS vectors to silence endogenous miRNAs in potato. Through 5′ RLM RACE (cDNA terminal rapid amplification), a dual luciferase assay, and virus-based microRNA silencing experiments, we confirmed that novel-miR1730-3p targets the 5′ UTR region of the *StXERICO1* gene, resulting in negative regulation of its expression (Figures 11–13), which means a new method for regulating the expression of the *XERICO* gene.

Ubiquitin-mediated protein degradation plays a key regulatory role in plant growth and development and is associated with plant hormone signaling [5]. StUBC has been identified as an E2 ubiquitin ligase [26]. Our yeast two-hybrid assay results clearly demonstrated an interaction between StXERICO1 and StUBC (Figure 14), suggesting that StXERICO1, functioning as an E3 ubiquitin ligase, requires combination with StUBC to perform its role.

In addition, StTLP is characterized as a Tubby-like protein, with 96% sequence similarity to AtTLP9 (At3g06380) [39]. The knockout mutant of AtTLP9 showed insensitivity to ABA, while transgenic plants overexpressing AtTLP9 exhibited heightened ABA sensitivity, indicating the involvement of AtTLP9 in ABA signaling pathways [39]. Our results demonstrated a clear interaction between StXERICO1 and StTLP (Figure 14), suggesting that StXERICO1 may be involved in the ABA signal transduction-dependent pathway. Potato StXERICO1 may also play a role in ABA homeostasis at the post-translational level, possibly through interaction with StTLP via ubiquitin/proteasome-dependent substrate-specific degradation, as reported in *Arabidopsis thaliana* [27]. The present validation results indicate the conservation of StXERICO1 function across different species, as its functionality in *Arabidopsis thaliana* is also confirmed in potato. This finding is significant for understanding the mechanistic role of this gene in plant biology and provides valuable references for molecular studies in related species.

In brief, the role of *StXERICO1* in regulating drought resistance in potato is summarized in Figure 15.

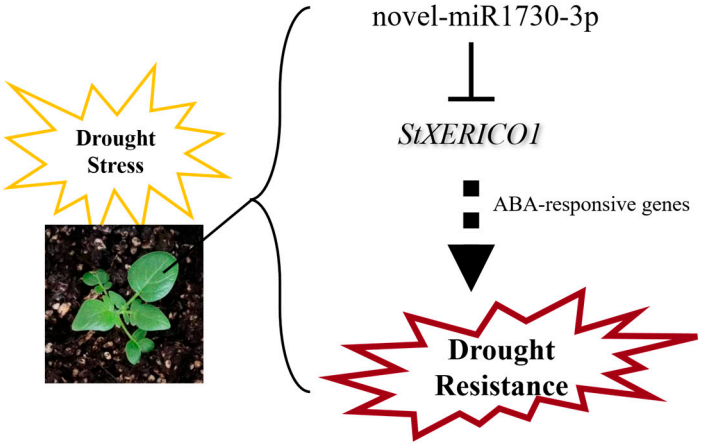

Figure 15. Molecular mechanism of the *StXERICO1* gene in enhancing drought resistance in potato.

5. Conclusions

The present study characterized a *XERICO* gene, *StXERICO1*, from a diploid potato, investigated its role in enhancing drought resistance and ABA accumulation, and identified its interaction with the miRNA novel-miR1730-3p, as well as its protein interactions with StUBC and StTLP. The expression of *StXERICO1* was highly responsive to drought, ABA treatment, and abiotic stresses in potato plants. Overexpression of the *StXERICO1* gene significantly enhanced drought resistance, increased ABA accumulation in transgenic potato and tobacco plants, and exhibited greater sensitivity to ABA treatment. This was associated with the upregulation of expression of ABA biosynthetic genes *NCED* and *CYP707A*. Furthermore, the results revealed that *StXERICO1* and its encoding protein interacted with miRNAs and other proteins. The miRNA novel-miR1730-3p targets the 5′ UTR region of the *StXERICO1* gene and negatively regulates *StXERICO1* expression. Additionally, StXERICO1 protein also interacts with StUBC and StTLP, suggesting that StXERICO1 might function on ABA homeostasis at the post-translational level. These findings elucidate the molecular mechanisms by which *StXERICO1*, a RING-H2 type E3 ubiquitin ligase, enhances drought resistance through increased ABA accumulation, how its expression is regulated by the miRNA, and how it exerts its function through interactions with other proteins. The results also provide a potential candidate gene for subsequent

precision molecular breeding aimed at improving crop drought resistance. By integrating *XERICO* into a molecular breeding program focused on developing resistant crop varieties, it may be possible to enhance abscisic acid (ABA) biosynthesis and improve overall crop stress tolerance mechanisms. This could lead to crops capable of maintaining yield stability in water-limited environments, thereby contributing to global food security.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14102305/s1, Table S1: All primer sequences used in this study. Table S2 List the miRNA targets of *StXERICO1*.

Author Contributions: Conceptualization, M.G.; formal analysis, J.Y. and Y.C.; funding acquisition, M.G. and C.L.; investigation, J.Y.; methodology, J.Y., Y.C. and L.W.; resources, M.G.; supervision, M.G. and C.L.; validation, J.Y.; writing—original draft preparation, J.Y., C.L. and M.G.; writing—review and editing J.Y., L.W., Y.C. and M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the National Natural Science Foundation of China (No. 32260459) and the open research program (No. YNPKF202202) of Yunnan Key Laboratory of Potato Biology, Yunnan Normal University.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We thank Yunnan Key Laboratory of Potato Biology, Yunnan Normal University for providing the gene knockout experimental protocol.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lesk, C.; Rowhani, P.; Ramankutty, N. Influence of extreme weather disasters on global crop production. *Nature* **2016**, *529*, 84–87. [CrossRef] [PubMed]
2. Feng, S.; Hao, Z.C.; Zhang, X.; Hao, F.H. Probabilistic evaluation of the impact of compound dry-hot events on global maize yields. *Sci. Total Environ.* **2019**, *689*, 1228–1234. [CrossRef] [PubMed]
3. Adekanmbi, T.; Wang, X.Q.; Basheer, S.; Nawaz, R.A.; Pang, T.Z.; Hu, Y.L.; Liu, S.Q. Assessing future climate change impacts on potato yields-A case study for Prince Edward Island, Canada. *Foods* **2023**, *12*, 1176. [CrossRef]
4. McNeilly, D.; Schofield, A.; Stone, S.L. Degradation of the stress-responsive enzyme formate dehydrogenase by the RING-type E3 ligase Keep on Going and the ubiquitin 26S proteasome system. *Plant Mol. Biol.* **2018**, *96*, 265–278. [CrossRef] [PubMed]
5. Sharma, S.; Prasad, A.; Sharma, N.; Prasad, M. Role of ubiquitination enzymes in abiotic environmental interactions with plants. *Int. J. Biol. Macromol.* **2021**, *181*, 494–507. [CrossRef]
6. Shu, K.; Yang, W. E3 ubiquitin ligases: Ubiquitous actors in plant development and abiotic stress responses. *Plant Cell Physiol.* **2017**, *58*, 1461–1476. [CrossRef]
7. Kelley, D.R. E3 ubiquitin ligases: Key regulators of hormone signaling in plants. *Mol. Cell Proteom.* **2018**, *17*, 1047–1054. [CrossRef]
8. Cho, S.K.; Ryu, M.Y.; Kim, J.H.; Hong, J.S.; Oh, T.R.; Kim, W.T.; Yang, S.W. RING E3 ligases: Key regulatory elements are involved in abiotic stress responses in plants. *BMB Rep.* **2017**, *50*, 393–400. [CrossRef]
9. Lim, S.D.; Yim, W.C.; Moon, J.C.; Kim, D.S.; Lee, B.M.; Jang, C.S. A gene family encoding RING finger proteins in rice: Their expansion, expression diversity, and co-expressed genes. *Plant Mol. Biol.* **2010**, *72*, 369–380. [CrossRef]
10. Stone, S.L.; Hauksdóttir, H.; Troy, A.; Herschleb, J.; Kraft, E.; Callis, J. Functional analysis of the RING-type ubiquitin ligase family of Arabidopsis. *Plant Physiol.* **2005**, *137*, 13–30. [CrossRef]
11. Tong, G.; Shen, H.M.; Cao, S.Q.; Xu, W.J.; Ma, X.J.; Cheng, Y.X. Identification of RING-H2 gene candidates related to wood formation in poplar. *Forests* **2019**, *10*, 698. [CrossRef]
12. Meng, X.W.; Liu, J.; Zhao, M.D. Genome-wide identification of RING finger genes in flax (*Linum usitatissimum*) and analyses of their evolution. *PeerJ* **2021**, *9*, e12491. [CrossRef] [PubMed]
13. Basso, M.F.; Ferreira, P.C.G.; Kobayashi, A.K.; Harmon, F.G.; Nepomuceno, A.L.; Molinari, H.B.C.; Grossi-de-Sa, M.F. MicroRNAs and new biotechnological tools for its modulation and improving stress tolerance in plants. *Plant Biotechnol. J.* **2019**, *17*, 1482–1500. [CrossRef] [PubMed]
14. Zhou, X.R.; Khare, T.; Kumar, V. Recent trends and advances in identification and functional characterization of plant miRNAs. *Acta Physiol. Plant* **2020**, *42*, 25. [CrossRef]
15. Zhang, S.S.; Wu, Y.Q.; Huang, X.; Wu, W.L.; Lyu, L.; Li, W.L. Research progress about microRNAs involved in plant secondary metabolism. *Int. J. Biol. Macromol.* **2022**, *216*, 820–829. [CrossRef]
16. Sun, X.; Lin, L.; Sui, N. Regulation mechanism of microRNA in plant response to abiotic stress and breeding. *Mol. Biol. Rep.* **2019**, *46*, 1447–1457. [CrossRef]

17. Pervaiz, T.; Amjid, M.W.; El-kereamy, A.; Niu, S.H.; Wu, H.X. MicroRNA and cDNA-microarray as potential targets against abiotic stress response in plants: Advances and prospects. *Agronomy* **2021**, *12*, 11. [CrossRef]
18. Ko, J.H.; Yang, S.H.; Han, K.H. Upregulation of an Arabidopsis RING-H2 gene, *XERICO*, confers drought tolerance through increased abscisic acid biosynthesis. *Plant J.* **2006**, *47*, 343–355. [CrossRef]
19. Brugière, N.; Zhang, W.J.; Xu, Q.Z.; Scolaro, E.J.; Lu, C.; Kahsay, R.Y.; Kise, R.; Trecker, L.; Williams, R.W.; Hakimi, S.; et al. Overexpression of RING domain E3 ligase ZmXerico1 confers drought tolerance through regulation of aba homeostasis. *Plant Physiol.* **2017**, *175*, 1350–1369. [CrossRef]
20. Kim, M.H.; Cho, J.S.; Park, E.J.; Lee, H.; Choi, Y.I.; Bae, E.K.; Han, K.H.; Ko, J.H. Overexpression of a poplar RING-H2 zinc finger, *Ptxerico*, confers enhanced drought tolerance via reduced water loss and ion leakage in *Populus*. *Int. J. Mol. Sci.* **2020**, *21*, 9454. [CrossRef]
21. Vonapartis, E.; Mohamed, D.; Li, J.R.; Pan, W.Q.; Wu, J.; Gazzarrini, S. CBF4/DREB1D represses *XERICO* to attenuate ABA, osmotic and drought stress responses in Arabidopsis. *Plant J.* **2022**, *110*, 961–977. [CrossRef] [PubMed]
22. Chen, Y.K.; Li, C.H.; Yi, J.; Yang, Y.; Lei, C.X.; Gong, M. Transcriptome response to drought, rehydration and re-dehydration in potato. *Int. J. Mol. Sci.* **2020**, *21*, 159. [CrossRef] [PubMed]
23. Lei, C.X.; Ye, M.W.; Li, C.H.; Gong, M. H_2O_2 participates in the induction and formation of potato tubers by activating tuberization-related signal transduction pathways. *Agronomy* **2023**, *13*, 1398. [CrossRef]
24. Ye, M.W.; Yao, M.F.; Li, C.H.; Gong, M. Salt and osmotic stress can improve the editing efficiency of CRISPR/Cas9- mediated genome editing system in potato. *PeerJ* **2023**, *11*, e15771. [CrossRef]
25. Zeng, D.E.; Hou, P.; Xiao, F.M.; Liu, Y.S. Overexpression of Arabidopsis *XERICO* gene confers enhanced drought and salt stress tolerance in rice (*Oryza Sativa* L.). *J. Plant Biochem. Biotechnol.* **2015**, *24*, 56–64. [CrossRef]
26. Pham, G.M.; Hamilton, J.P.; Wood, J.C.; Burke, J.T.; Zhao, H.; Vaillancourt, B.; Ou, S.; Jiang, J.; Buell, C.R. Construction of a chromosome-scale long-read reference genome assembly for potato. *GigaScience* **2020**, *9*, giaa100. [CrossRef]
27. Zhang, H.; Sun, X.P.; Dai, M.Q. Improving crop drought resistance with plant growth regulators and rhizobacteria: Mechanisms, applications, and perspectives. *Plant Commun.* **2021**, *3*, 100228. [CrossRef]
28. Bouaziz, D.; Pirrello, J.; Charfeddine, M.; Hammami, A.; Jbir, R.; Dhieb, A.; Bouzayen, M.; Gargouri-Bouzid, R. Overexpression of *StDREB1* transcription factor increases tolerance to salt in transgenic potato plants. *Mol. Biotechnol.* **2013**, *54*, 803–817. [CrossRef] [PubMed]
29. Lehretz, G.G.; Sonnewald, S.; Lugassi, N.; Granot, D.; Sonnewald, U. Future-Proofing potato for drought and heat tolerance by overexpression of Hexokinase and SP6A. *Front. Plant Sci.* **2021**, *11*, 614534. [CrossRef]
30. Zhu, J.K. Abiotic Stress Signaling and Responses in Plants. *Cell* **2016**, *167*, 313–324. [CrossRef]
31. Yang, W.; Liu, X.D.; Chi, X.J.; Wu, C.A.; Li, Y.Z.; Song, L.L.; Liu, X.M.; Wang, Y.F.; Wang, F.W.; Zhang, C.; et al. Dwarf apple MbDREB1 enhances plant tolerance to low temperature, drought, and salt stress via both ABA-dependent and ABA-independent pathways. *Planta* **2011**, *233*, 219–229. [CrossRef] [PubMed]
32. Ji, K.; Kai, W.; Zhao, B.; Sun, Y.; Yuan, B.; Dai, S.; Li, Q.; Chen, P.; Wang, Y.; Pei, Y.; et al. SlNCED1 and SlCYP707A2: Key genes involved in ABA metabolism during tomato fruit ripening. *J. Exp. Bot.* **2014**, *65*, 5243–5255. [CrossRef] [PubMed]
33. Hao, D.H.; Gong, M. The progress of miRNA action mechanism. *Genom. Appl. Biol.* **2020**, *39*, 3647–3657. [CrossRef]
34. Tang, Y.; Wang, F.; Zhao, J.P.; Xie, K.; Hong, Y.G.; Liu, Y.L. Virus-based microRNA expression for gene functional analysis in plants. *Plant Physiol.* **2010**, *153*, 632–641. [CrossRef]
35. Fang, L.; Wei, X.Y.; Liu, L.Z.; Zhou, L.X.; Tian, Y.P.; Geng, C.; Li, X.D. A tobacco ringspot virus-based vector system for gene and microRNA function studies in cucurbits. *Plant Physiol.* **2021**, *187*, 1835. [CrossRef]
36. Jiang, L.L.; Du, Z.H.; Zhang, G.Z.; Wang, T.; Jin, G.H. Advances in RNA-Silencing-Related resistance against viruses in potato. *Genes* **2022**, *13*, 731. [CrossRef]
37. Luo, H.Y.; Yang, J.W.; Liu, S.Y.; Li, S.G.; Si, H.J.; Zhang, N. Control of plant height and lateral root development via Stu-miR156 regulation of SPL9 transcription factor in potato. *Plants* **2024**, *13*, 723. [CrossRef]
38. Yang, J.W.; Zhang, N.; Bai, J.P.; Duan, X.Q.; Zhang, L.H.; Liu, S.Y.; Tang, X.; Jin, X.; Li, S.G.; Si, H.J. Stu-miR827-targeted *StWRKY48* transcription factor negatively regulates drought tolerance of potato by increasing leaf stomatal density. *Int. J. Mol. Sci.* **2022**, *23*, 14805. [CrossRef]
39. Lai, C.P.; Lee, C.L.; Chen, P.H.; Wu, S.H.; Yang, C.C.; Shaw, J.F. Molecular analyses of the Arabidopsis TUBBY-like protein gene family. *Plant Physiol.* **2004**, *134*, 1586–1597. [CrossRef]