



Article

Exploring Candidate Genes and Regulatory Mechanisms for Salt–Alkali Tolerance in Cucumber

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Abstract: Soil salinization is one of the main problems faced by modern agricultural production, especially cucumber production. This study screened the salt–alkali-tolerant cultivar ‘D1909’ and the salt–alkali-sensitive cultivar ‘D1604’ from 32 different cucumber ecological types on the basis of morphological indicators and salt–alkali indices combined with relevant physiological and biochemical indices. By performing a transcriptome metabolome analysis, the key gene *CsSRG1*, which is responsive to salt–alkali stress in cucumber, was screened, and its function was verified. The role of *CsSRG1* in reducing salt–alkali stress in cucumber was clarified, and the mechanism of salt–alkali tolerance in cucumber was preliminarily explored. This study provides germplasm resources for cucumber salt–alkali tolerance breeding and a theoretical basis for the effective use of saline alkali soil to achieve high quality and high yield in other crops.

Keywords: cucumber; salt–alkali stress; transcriptomic and metabolomic analysis; *CsSRG1*; functional analysis



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1. Introduction

Cucumber (*Cucumis sativus* L.) originated in India and belongs to the Cucurbitaceae family. It is an important economic crop that is widely cultivated worldwide [1]. Since its introduction to China, the planting area of cucumber has constantly expanded, and the plant has become very popular among the general public. However, cucumber plants have strict requirements for environmental conditions and prefer neutral to acidic soil, making them susceptible to the effects of salt and alkali stress. Due to the shallow root system and weak absorption capacity, the suitable soil humidity is 60–90%, and the suitable relative air humidity is 60–80%. The salinization of soil can lead to a decrease in the abundance of soil animals and microorganisms, a decrease in plant chlorophyll content, and a decrease in photosynthetic and transpiration rates. This can directly result in a series of morphological and physiological changes in plants, such as root rot, plant wilting, leaf yellowing, and insufficient fruit bearing, severely affecting cucumber yield and product quality [2]. In recent years, with the intensification of soil salinization, cucumber tolerance to salt–alkali stress has gradually become a popular research topic. The screening and cultivation of cucumber varieties with salt–alkali tolerance is an effective way to reduce salt damage during production [3], and the use of saline soil for cucumber plants should receive increased attention.

ABC transporters, also known as adenosine triphosphate-binding cassette transporters (ATP-binding cassette transporters), participate in a series of plant life activities by transporting various substrates. Many ABC transporters have been shown to be involved in the plant response to salt–alkali stress. The overexpression of the *AtSIA1* gene can increase the tolerance of Arabidopsis to salt stress [4]. The overexpression of *AtABC1* in Arabidopsis can enhance plant resistance to salt stress and low temperatures [5]. In addition, in wheat, *TaABC1L* can respond to stress, such as high salt concentrations and low temperature [6].

The *Abc1* gene in rice (*Oryza sativa*) is expressed mainly in the leaves and is regulated by various abiotic stress factors, including H₂O₂, ABA, low temperatures, and high levels of salt [7]. In summary, there have been many studies on the role of ABC family genes in the response to salt stress in cucumber, but the function of these genes in the resistance to salt–alkali stress in cucumber has not been determined.

SRG1-related proteins can promote the biosynthesis of flavonoids, ethylene, alkaloids, and various hormones in plants, effectively enhancing plant resistance and performing very important biological functions. The *SRG5* gene does not participate in the response to high-temperature stress but is involved in the early response to dry conditions and salt stress and has a positive regulatory effect [8]. The overexpression of the *MaSRG1* gene has a positive effect on the resistance of wild-type tobacco leaves to phytophthora capsici and botrytis cinerea [9]. Several animal experiments have shown that the SRG1 protein, a type of synaptic binding protein, can play a nutritional and supportive role in the brain, maintaining the normal physiological function of neurons. The role of the SRG1 protein in cucumber has not been reported to date. Identifying the function of SRG1 proteins in cucumber, understanding their role in metabolic pathways, and discovering the unknown regulatory mechanisms will thus improve the understanding of the physiological role of SRG1 proteins in cellular metabolism.

This study screened the salt–alkali-tolerant strain ‘D1909’ and the salt–alkali-sensitive strain ‘D1604’ through morphogenesis, salt–alkali level identification, and salt–alkali index analysis of cucumber plants under salt–alkali stress. Relevant physiological and biochemical indicators were tested in the leaves of the plants, and significant physiological differences were detected among the different cucumber varieties under salt–alkali stress. The transcriptional metabolism analysis of ‘D1909’ and ‘D1604’ revealed the differentially expressed gene (DEG) *CsSRG1*, suggesting that this gene may be key for cucumber salt–alkali stress tolerance. The cloning and functional validation of the *CsSRG1* gene were assessed to explore whether *CsSRG1* can actively respond to salt–alkali stress and play a role in resisting salt–alkali damage in cucumber by measuring its expression levels in different organs of cucumber plants and measuring physiological indicators in cucumber plants overexpressing this gene under salt–alkali stress. These findings lay a foundation for the study of the molecular mechanisms of cucumber salt–alkali tolerance and the cultivation of new salt–alkali-tolerant varieties.

2. Materials and Methods

2.1. Experimental Materials

In this study, 32 cucumber cultivars of different ecological types were selected as experimental materials (Table 1), and the seeds were obtained from the College of Horticulture and Landscape Architecture of Heilongjiang Bayi Agricultural University in Daqing city, China (45°46′ N, 124°19′ E).

Table 1. Cucumber ecological type.

Code	Accession	Ecotype	Code	Accession	Ecotype
1	C17-114	South China type	17	D1104-2-4	South China type
2	C18-039	South China type	18	JZ6-1-2	South China type
3	C18-062	South China type	19	D1909	South China type
4	C18-069	South China type	20	D1158-1	South China type
5	C18-071	South China type	21	B1106-1	North China type
6	C18-073	South China type	22	D1604	North China type
7	C18-102	South China type	23	D0328-3	North China type
8	C18-106	South China type	24	D0328-19	North China type
9	C18-301	South China type	25	B1103-1	Pickled type
10	C18-303	South China type	26	C19-01	Pickled type
11	DN 808	South China type	27	C19-68	Pickled type
12	DN 812	South China type	28	C19-69	Pickled type

Table 1. Cont.

Code	Accession	Ecotype	Code	Accession	Ecotype
13	DN 816	South China type	29	C19-100	Pickled type
14	D0528-4	South China type	30	C19-105	Pickled type
15	T0351	South China type	31	C19-213	Pickled type
16	T649-1	South China type	32	DN 809	Pickled type

2.2. Experimental Material Treatment

The saline–alkaline soil in the Daqing area is sulfate soda saline–alkaline soil. To simulate sulfate soda saline–alkali soil, a mixed saline–alkali solution consisting of NaCl, Na₂CO₃, NaHCO₃, and Na₂SO₄ at a molar ratio of 1:1:9:9 was prepared. The total salt concentration was calculated based on the Na ion content of 80 mmol·L^{−1}, and the pH of the solution was 8.9. This salt was used to screen cucumber cultivars that were tolerant to salt–alkali conditions [10].

A bucket with a diameter of 30 cm and a height of 40 cm was selected for potting. The soil used was simulated sulfate soda saline–alkali soil prepared with sterile soil and a saline–alkali solution. The sterile soil was purchased from the Shengbinghe material distribution office in Daqing city. The salt content of the soil used for potted plant screening was 3.3‰, and the pH was 8.9 ± 0.5. The soil was stirred evenly, and each barrel in the treatment group was filled with 20 kg of salt–alkali soil, while barrels in the control group were filled with 20 kg of sterile soil. Ten varieties were sown per barrel at a depth of 2 cm. The greenhouse temperature was maintained at 25 °C during the day for 16 h and at 18 °C at night for 8 h, with a relative humidity of 70%. The plant height, stem diameter, leaf area, fresh weight, and dry weight were measured during the three-leaf and one-heart stage. The functional leaves were cut, mixed, and measured to assess physiological indicators. Three-leaf and one-heart-stage leaves were frozen in liquid nitrogen and stored at −80 °C for subsequent use in gene expression measurements. The experiment was repeated three times.

Salt–alkali tolerance index: the membership function method [11] was used to evaluate the salt–alkali tolerance of each variety.

$$X_i = \frac{1}{n} \sum_{i=1}^n \frac{(I - I_{min})}{(I_{max} - I_{min})}$$

n represents the number of indicators to be tested, *I* is the average value of a certain indicator of the experimental variety, and *I*_{max} and *I*_{min} are the maximum and minimum values of the *I* indicator of the experimental variety, respectively [12]. The membership function values of each indicator were summed in order to calculate the average value as the salt–alkali tolerance index. The salt–alkali tolerance was determined based on the salt–alkali damage index and the classification criteria in Table 2. This study evaluated the salt–alkali tolerance of cucumber plants. According to the range of salt–alkali indices, 32 cucumber materials were divided into 5 groups, with a salt–alkali index ≥80% indicating very high salt–alkali tolerance, a salt–alkali index in the range of 60–80% indicating high salt–alkali tolerance, a salt–alkali index in the range of 40–60% indicating moderate salt–alkali tolerance, a salt–alkali index in the range of 20–40% indicating sensitivity, and a salt–alkali index <20% indicating extreme sensitivity.

Table 2. Salt–alkali tolerance corresponding to salt–alkali damage index.

Level	Salt Damage Index/%	Salt Tolerance
1	Salt damage index ≥80%	High salt–alkali tolerance
2	Salt damage index 60–80%	Salt–alkali tolerance
3	Salt damage index 40–60%	Medium salt–alkali tolerance
4	Salt damage index 20–40%	Salt–alkali sensitivity
5	Salt damage index <20%	High salt–alkali sensitivity

2.3. Measurement of Physiological and Biochemical Indicators

A 0.5 g fresh sample of cucumber leaves was collected, after which 3 mL of phosphate buffer (pH = 7.8) was added, and the mixture was ground in an ice bath and centrifuged for 20 min at 10,500 rpm. This sample was stored at 4 °C and subsequently used for the measurement of physiological and biochemical indices.

POD activity was determined via the guaiacol method with minor modifications. To prepare the reaction mixture, 112 µL of guaiacol was added to 200 mL of phosphate buffer (pH = 6.0) and heated until it was dissolved sufficiently; then, 19 µL of H₂O₂ was added (30%). The obtained solution was used as the reaction mixture and stored at 4 °C. A total of 20 µL of the reaction mixture was added to 3 mL of reaction liquid, with 20 µL of phosphate buffer added to 3 mL of reaction liquid as a control. The samples were placed into an ultraviolet–visible spectrophotometer (Shimadzu, Japan), and the optical density (OD) was recorded at 470 nm/min (OD values for 0, 1, 2, and 3 min) [13].

SOD activity was determined via the NBT method with minor modifications. The reaction mixture was prepared with H₂O, phosphate buffer, Met, NBT, EDTA-Na₂ and lactochrome at a ratio of 5:30:6:6:6:6. Then, 50 µL of the reaction mixture was added to 3 mL of reaction liquid, with 50 µL of phosphate buffer added to 3 mL of reaction liquid as a control. The control sample was divided into two groups: control group 1 was incubated in the dark, and control group 2 was incubated in normal light. The absorbance was determined with an ultraviolet–visible spectrophotometer (Shimadzu, Japan) at OD560 [14].

CAT was performed according to the H₂O₂ method with minor modification. Then, 50 mL of H₂O₂ (0.1 mol/L) was added to 200 mL of phosphate buffer (pH = 7.0). The obtained solution was used as the reaction mixture and stored at 4 °C. One hundred microliters of reaction mixture was added to 3 mL of reaction liquid, with 100 µL of phosphate buffer added to 3 mL of reaction liquid as a control. The samples were placed into an ultraviolet–visible spectrophotometer (Shimadzu, Japan), and the OD was recorded at 240 nm/min (OD values for 0, 1, 2, and 3 min) [15].

Chlorophyll content: A freshly mixed sample (0.3 g) was placed in a prepared mortar, and 2–3 mL of calcium carbonate, quartz sand, and 95% ethanol were added. The mixture was ground until the sample appeared green and homogeneous, 95% ethanol was added, and the mixture was then ground until the homogenate turned white. The mixture was left to stand for 5 min. The ground homogenate was filtered and diluted in a 25 mL brown volumetric flask. The chlorophyll extraction solution was measured using a spectrophotometer, with 95% ethanol as the blank control, and the absorbance was measured at 470, 649, and 665 nm.

2.4. Transcriptomic and Metabolomic Sample Collection

‘D1909’ and ‘D1604’ cucumber plants at the three-leaf one-heart stage were used as experimental materials. We randomly selected 20 plants of each variety from the saline–alkali treatment group and 20 plants from the control group and collected mixed leaf samples. The samples were named as follows: ‘D1909’ transcriptomic control: SAT_CK1, SAT_CK2, and SAT_CK3; transcriptome processing: SAT1, SAT2, and SAT3; metabolomics control: DSATCK1, DSATCK2, DSATCK3, DSATCK4, DSATCK5, and DSATCK6; metabolome processing: DSAT1, DSAT2, DSAT3, DSAT4, DSAT5, and DSAT6; ‘D1604’ transcriptomic control: NSAT_CK1, NSAT_CK2, and NSAT_CK3; transcriptome processing: NSAT1, NSAT2, and NSAT3; metabolomic control: DNSATCK1, DNSATCK2, DNSATCK3, DNSATCK4, DNSATCK5, and DNSATCK6; and metabolome processing: DNSAT1, DNSAT2, DNSAT3, DNSAT4, DNSAT5, and DNSAT6. All the sampled leaves were immediately placed in liquid nitrogen for 5 min, stored at −80 °C, and subsequently sent to Shanghai Meiji Biological Company for analysis (Shanghai, China).

2.5. Total RNA Extraction and Sequencing

Total RNA was extracted from the tissue according to the manufacturer’s instructions using TRIzol[®] Reagent. Then, RNA quality was determined using a 5300 Bioanalyzer

(Agilent, Santa Clara, CA, USA), and the RNA integrity number (RIN) was determined using an ND-2000 (NanoDrop Technology, Thermo Fisher Scientific, Waltham, MA, USA). A reverse transcription kit was used to transcribe total RNA to cDNA. The RNA-seq transcriptome library was prepared with the Illumina® Stranded mRNA Prep Ligation Kit (San Diego, CA, USA) using 1 µg of total RNA. The paired-end RNA-seq library was sequenced with a NovaSeq 6000 sequencer (2 × 150 bp read length).

2.6. mRNA Sequence Data Processing

The sequencing data were filtered using Fastp and default standards. Low-quality reads and adapter segments were removed from the original data, and HISAT2 was used to align the sequencing data to the cucumber reference genome after quality control. Quantitative analysis of the gene and transcript expression levels was conducted using RSEM (V1.3.3) software. DEGs were identified through different comparisons using DESeq2. The negative binomial distribution test and log-corrected *p*-value (*padj*<) were used with a standard of 0.052 (fold change (FC)) > 0. Genes with log 2FC > 0 and log 2FC < 0 were identified as upregulated and downregulated DEGs, respectively. DEGs enriched in modules were subjected to GO and KEGG pathway enrichment analyses in relation to phenotype. Significantly enriched GO terms in the biological process (BP) category and KEGG pathways were identified using *p* < 0.05 as the criterion.

2.7. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–MS analysis was performed using an Agilent 8890B gas chromatograph coupled with an Agilent 5977B mass selective detector at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The instrument was equipped with an inert electron impact (EI) ionization source with an ionization voltage of 70 eV (Agilent, Santa Clara, CA, USA). The samples were separated with a DB-5MS (40 m × 0.25 mm × 0.25 µm) capillary column, using 99.999% helium as the carrier gas at a constant flow rate (1 mL/min). The GC column temperature was programmed to hold at 60 °C for 30 s and increase to 310 °C at a rate of 8 °C per minute for 6 min. The sample injection volume was 1 µL, and the samples were introduced in splitting mode (15:1) with an inlet temperature of 260 °C. The mass spectrometry conditions were as follows: the ion source temperature was 230 °C, and the quadrupole temperature was 150 °C. The scanning mode was full scan mode, the quality scanning range was 50–500 *m/z*, and the scanning frequency was 3.2 scans/s.

2.8. Quality Control

To evaluate the stability of the analytical system during the run-on process, a quality control (QC) sample was prepared for the experiment. The QC samples were prepared by mixing all the test samples and were treated in the same way as the test samples were. During instrument testing, a QC sample was inserted every 5–15 samples. The repeatability of the results for the QC samples reflected the stability of the instrument throughout the analysis process. Moreover, this approach could also be used to identify variables with large variations in the analysis system to ensure the reliability of the results.

2.9. GC–MS Data Analysis

The raw mass spectrometry data obtained via GC–MS were preprocessed using MassHunter workstation Quantitative Analysis (version v10.0.707.0) software, and a three-dimensional data matrix was exported in CSV format. The information in this three-dimensional matrix included sample information, metabolite name, and mass spectral response intensity. Internal standard peaks, as well as any known false-positive peaks (including noise, column bleeds, and derivatized reagent peaks), were removed from the data matrix, as were the deredundant and pooled peaks. At the same time, the metabolites were identified by searching databases, and the main databases used were public databases such as NIST (version 2017), Fiehn (version 2013), and MS-DIAL (version 2021). The data matrix obtained by searching the database was uploaded to the Majorbio cloud

platform (<https://cloud.majorbio.com>, accessed on 25 November 2023) for data analysis. First, the data matrix was preprocessed as follows: at least 80% of the metabolic features detected in any set of samples were retained. After filtering, the minimum metabolite value was estimated for specific samples with metabolite levels below the lower limit of quantification, and each metabolic signature was normalized to the sum. To reduce the errors caused by sample preparation and instrument instability, the response intensities of the sample mass spectrometry peaks were normalized, using the sum normalization method to obtain the normalized data matrix. Moreover, variables with a relative standard deviation (RSD) >30% were excluded from the QC samples, and the values were log10-transformed to obtain the final data matrix for subsequent analysis. The R package “ropls” (version 1.6.2) was subsequently used to perform principal component analysis (PCA), orthogonal least-partial-squares discriminant analysis (OPLS-DA), and 7-cycle interactive validation to evaluate the stability of the model. The metabolites with VIP > 1 and $p < 0.05$ were determined to be significantly differentially abundant metabolites based on the variable importance in the projection (VIP) obtained using the OPLS-DA model and the p -value generated using the Student t test. The differentially abundant metabolites between the two groups were mapped to biochemical pathways through metabolic enrichment and pathway analysis performed via the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<http://www.genome.jp/kegg/>, accessed on 25 November 2023). These metabolites can be classified according to the pathways they are involved in or the functions they perform. Enrichment analysis was used to analyze whether a group of metabolites appeared within a functional node. The principle applied was that the annotation of a single metabolite could be used to annotate a group of metabolites. The Python package “scipy.stats” (<https://docs.scipy.org/doc/scipy/>, accessed on 25 November 2023) was used to perform enrichment analysis to obtain the most relevant biological pathways for the experimental treatments.

2.10. Cloning of CsSRG1

The full-length coding sequence (CDS) of CsSRG1 was obtained via BLAST alignment with the cucumber genome database. Primer Premier 5.0 software was used to design primers (CsSRG1-F and CsSRG1-R; Table S1) for the full-length coding region of the cloned gene. With D1909 cDNA as a template, PCR amplification was performed as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 57.0 °C for 30 s, and 72 °C for 30 s; and 72 °C for 10 min. The amplified PCR products were detected via agarose gel electrophoresis, and a colloid recovery kit (TransGen Biotech, Beijing, China) was used to recover the target bands. The recovered fragments were subsequently inserted into the T3 vector (TransGen Biotech). After the reaction, the sample was transformed into *Escherichia coli* DH5 α competent cells via thermal shocking, followed by overnight incubation on LB agar plates containing X-Gal, IPTG, and Amp at 37 °C. Single white bacterial colonies were isolated and sent for sequencing (Qing Ke).

2.11. Expression Pattern Analysis

Online software (<https://www.genscript.com/tools/real-time-pcr-tagman-primer-design-tool>, accessed on 25 November 2023) was used for primer design, and the primers used are shown in Table S1. Relative quantitation of gene expression was performed using CsEF1 α (GenBank accession number: XM_004138916) as a control [16]. Three biological replicates and three technical replicates were performed. The $2^{-\Delta\Delta CT}$ method was used to analyze the real-time qRT-PCR results [17].

2.12. Genetic Transformation of Cucumber by CsSRG1

With full-length primers and the CsSRG1-F, CsSRG1-R, and pEASYT3-CsSRG1 plasmids serving as templates, the target gene was amplified via PCR. The vector PCXSN-1250 was digested via XcmI [18]. T4 ligase was used to ligate the target fragment in the vector, and the vector was subsequently transformed into *E. coli*. The pCXSN-CsSRG1 construct

was confirmed via PCR and sequencing. The constructed expression vectors were subsequently transferred into *Agrobacterium tumefaciens* LBA4404 via the freeze–thaw method in order to infect cucumber cotyledon nodes. Genetic transformation technology was used for cucumber, and MS media supplemented with 1.0 mg/L glyphosate were screened and used as the differentiation media. After 20 days, the cotyledon nodes began to differentiate, and the differentiated buds grew. The buds were then cut off and transferred to rooting media. After the main roots and fibrous roots grew, they were transplanted and domesticated in an incubator to obtain T0 transgenic cucumber plants [19]. The DNA of the transgenic cucumber plants was extracted using the CTAB method, and the sequence of the pCXSN vector was used for primer design. The transgenic plants were identified via PCR and qPCR (T₀ plants).

2.13. Statistical Analysis

All data measurements were replicated at least three times. The data were subjected to statistical analyses using the data processing system Origin 8.0 and DPS 9.5. The data are expressed as the means $\pm$ SDs. The significance of the differences between the treatment and control groups was confirmed using the Student *t* test. The data were analyzed by analysis of variance ($p < 0.05$ and $p < 0.001$ indicate significant and extremely significant differences, respectively).

3. Results and Analysis

3.1. Screening of Germplasm Resources for Salt–Alkali-Tolerant Cultivars

The plant height, stem diameter, leaf area, and fresh and dry mass of the cucumber plants under salt–alkali stress were measured during the three-leaf stage. The results are shown in Table 3. Under salt–alkali stress, all the indicators of the 32 cultivars exhibited certain differences. The height of the ‘D1909’ plants was 13.33 cm, the stem diameter was 0.39 cm, the leaf area was 56.00 cm², the fresh mass of the whole plant was 4.05 g, and the dry mass was 0.36 g; these values were significantly or extremely significantly greater than those of the other 31 cultivars. The plant height of ‘D1604’ was 7.62 cm, the leaf area was 0.20 cm², the fresh mass of the whole plant was 2.41 g, and the dry mass was 0.22 g; these values were significantly or extremely significantly lower than those of the other cultivars. The stem diameter was 0.20 cm, which was only slightly greater than that of ‘D1104-2-4’.

Table 3. Cucumber germplasm resources and their main agronomic traits.

Code	Plant Height (cm)	Stem Diameter (cm)	Leaf Area (cm ²)	Fresh Mass (g)	Dry Mass (g)	Salt–Alkali Level	Salt–Alkali Index	Salt–Alkali Tolerance
1	9.24 $\pm$ 0.34	0.33 $\pm$ 0.02	35.24 $\pm$ 2.16	3.17 $\pm$ 0.29	0.28 $\pm$ 0.02	3	46.14%	medium salt–alkali tolerance
2	9.87 $\pm$ 0.26	0.36 $\pm$ 0.03	38.16 $\pm$ 3.12	3.65 $\pm$ 0.26	0.32 $\pm$ 0.03	2	64.41%	salt–alkali tolerance
3	10.36 $\pm$ 0.51	0.28 $\pm$ 0.02	42.00 $\pm$ 2.22	3.36 $\pm$ 0.24	0.29 $\pm$ 0.02	3	52.84%	medium salt–alkali tolerance
4	9.70 $\pm$ 0.53	0.28 $\pm$ 0.02	22.46 $\pm$ 2.13	3.48 $\pm$ 0.31	0.34 $\pm$ 0.03	3	48.16%	medium salt–alkali tolerance
5	8.95 $\pm$ 0.41	0.31 $\pm$ 0.03	30.18 $\pm$ 3.18	3.25 $\pm$ 0.22	0.30 $\pm$ 0.03	3	44.21%	medium salt–alkali tolerance
6	8.43 $\pm$ 0.26	0.24 $\pm$ 0.02	32.04 $\pm$ 2.84	2.78 $\pm$ 0.23	0.26 $\pm$ 0.02	4	25.32%	salt–alkali sensitivity
7	9.28 $\pm$ 0.39	0.22 $\pm$ 0.03	36.84 $\pm$ 2.47	3.49 $\pm$ 0.34	0.30 $\pm$ 0.03	3	43.46%	medium salt–alkali tolerance
8	10.35 $\pm$ 0.44	0.36 $\pm$ 0.03	36.93 $\pm$ 2.83	3.07 $\pm$ 0.28	0.31 $\pm$ 0.03	3	56.90%	medium salt–alkali tolerance
9	9.85 $\pm$ 0.42	0.24 $\pm$ 0.02	40.35 $\pm$ 3.62	3.38 $\pm$ 0.36	0.29 $\pm$ 0.02	3	46.56%	medium salt–alkali tolerance
10	10.50 $\pm$ 0.34	0.27 $\pm$ 0.03	38.87 $\pm$ 2.85	3.39 $\pm$ 0.25	0.30 $\pm$ 0.03	3	52.42%	medium salt–alkali tolerance
11	11.45 $\pm$ 0.38	0.33 $\pm$ 0.03	40.17 $\pm$ 3.32	3.60 $\pm$ 0.32	0.31 $\pm$ 0.03	2	66.18%	salt–alkali tolerance
12	11.88 $\pm$ 0.39	0.26 $\pm$ 0.02	44.27 $\pm$ 3.14	3.67 $\pm$ 0.22	0.33 $\pm$ 0.03	2	67.03%	salt–alkali tolerance
13	9.03 $\pm$ 0.27	0.34 $\pm$ 0.03	49.12 $\pm$ 2.77	2.60 $\pm$ 0.24	0.23 $\pm$ 0.02	3	40.06%	medium salt–alkali tolerance
14	10.18 $\pm$ 0.36	0.25 $\pm$ 0.03	47.38 $\pm$ 2.87	3.11 $\pm$ 0.27	0.28 $\pm$ 0.02	3	47.90%	medium salt–alkali tolerance
15	11.35 $\pm$ 0.53	0.25 $\pm$ 0.02	38.66 $\pm$ 3.05	3.85 $\pm$ 0.28	0.35 $\pm$ 0.03	2	66.12%	salt–alkali tolerance
16	11.35 $\pm$ 0.41	0.31 $\pm$ 0.02	49.50 $\pm$ 3.11	3.77 $\pm$ 0.34	0.34 $\pm$ 0.02	2	75.52%	salt–alkali tolerance
17	10.75 $\pm$ 0.38	0.18 $\pm$ 0.03	41.87 $\pm$ 3.43	3.71 $\pm$ 0.29	0.33 $\pm$ 0.03	3	54.59%	medium salt–alkali tolerance
18	9.58 $\pm$ 0.28	0.32 $\pm$ 0.03	44.34 $\pm$ 2.73	3.29 $\pm$ 0.36	0.29 $\pm$ 0.03	3	54.38%	medium salt–alkali tolerance
19	13.33 $\pm$ 0.34	0.39 $\pm$ 0.03	56.00 $\pm$ 2.73	4.05 $\pm$ 0.37	0.36 $\pm$ 0.03	1	100.00%	high salt–alkali tolerance
20	11.13 $\pm$ 0.44	0.32 $\pm$ 0.03	51.38 $\pm$ 3.25	3.45 $\pm$ 0.32	0.31 $\pm$ 0.02	2	68.57%	salt–alkali tolerance
21	12.07 $\pm$ 0.54	0.32 $\pm$ 0.03	50.22 $\pm$ 2.67	3.87 $\pm$ 0.32	0.34 $\pm$ 0.03	1	80.62%	high salt–alkali tolerance

Table 3. Cont.

Code	Plant Height (cm)	Stem Diameter (cm)	Leaf Area (cm ²)	Fresh Mass (g)	Dry Mass (g)	Salt-Alkali Level	Salt-Alkali Index	Salt-Alkali Tolerance
22	7.62 ± 0.29	0.20 ± 0.03	20.40 ± 3.12	2.41 ± 0.27	0.22 ± 0.02	5	1.90%	high salt-alkali sensitivity
23	8.42 ± 0.19	0.25 ± 0.02	31.55 ± 2.85	2.70 ± 0.29	0.24 ± 0.02	4	22.13%	salt-alkali sensitivity
24	9.91 ± 0.33	0.24 ± 0.03	33.28 ± 2.71	3.43 ± 0.25	0.31 ± 0.03	3	46.27%	medium salt-alkali tolerance
25	11.29 ± 0.37	0.36 ± 0.03	37.61 ± 2.82	3.75 ± 0.27	0.34 ± 0.03	2	73.15%	salt-alkali tolerance
26	12.03 ± 0.22	0.28 ± 0.03	45.04 ± 3.45	3.96 ± 0.32	0.35 ± 0.03	2	76.29%	salt-alkali tolerance
27	11.57 ± 0.34	0.33 ± 0.02	40.80 ± 3.32	3.64 ± 0.24	0.33 ± 0.03	2	70.30%	salt-alkali tolerance
28	11.77 ± 0.40	0.29 ± 0.03	39.55 ± 2.61	3.56 ± 0.34	0.32 ± 0.03	2	64.08%	salt-alkali tolerance
29	12.03 ± 0.38	0.32 ± 0.03	42.19 ± 2.62	3.88 ± 0.33	0.35 ± 0.03	2	77.52%	salt-alkali tolerance
30	9.96 ± 0.35	0.24 ± 0.03	38.76 ± 3.26	2.98 ± 0.28	0.26 ± 0.02	4	36.89%	salt-alkali sensitivity
31	10.21 ± 0.27	0.26 ± 0.03	41.92 ± 3.31	3.45 ± 0.36	0.31 ± 0.02	3	54.32%	medium salt-alkali tolerance
32	9.78 ± 0.41	0.33 ± 0.02	42.00 ± 3.09	3.58 ± 0.23	0.32 ± 0.02	2	62.54%	salt-alkali tolerance

To investigate the differences in the performance of the cucumber cultivars ‘D1909’ and ‘D1604’ under salt-alkali stress, relevant physiological and biochemical indicators were tested for the salt-alkali-tolerant cultivar ‘D1909’ and the salt-alkali-sensitive cultivar ‘D1604’. As shown in Figure 1, the chlorophyll content of ‘D1909’ and ‘D1604’ after exposure to salt-alkali stress was extremely significantly lower than that of the control group, and the chlorophyll content of ‘D1909’ was 0.22 mg/g greater than that of ‘D1604’ (Figure 1A). The CAT content of ‘D1909’ was extremely significantly lower than that of the control group under salt-alkali stress, while the CAT content of ‘D1604’ was not significantly different from that of the control group (Figure 1B). Under salt-alkali stress, the SOD content tended to increase in both ‘D1909’ and ‘D1604’, the SOD content in ‘D1909’ was significantly greater than that in the control group ($78.35 \text{ U} \cdot \text{g}^{-1} \text{ FW}$), while that in D1604 was not significantly different from that in the control group (Figure 1C). The POD activity in the ‘D1909’ salt-alkali stress group was extremely significantly greater than that in the control group (by 1.72-fold). The difference between the ‘D1604’ cultivar in the salt-alkali stress group and the control group was not significant, but its POD activity was slightly lower than that of the control group, which was $8.60 \triangle \text{OD}_{470} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \text{ FW}$ (Figure 1D).

3.2. Transcriptome Analysis of ‘D1909’ and ‘D1604’

To investigate the differences in gene expression between the ‘D1604’ and ‘D1909’ cucumber plants in the salt-alkali stress group and the control group, RNA-seq analysis was performed on the leaves. All the statistical analyses were conducted to provide general information for the project. Here, transcriptome analysis of 12 samples was completed, each containing three replicates, for a total of 83.07 Gb of clean data. The amount of clean data in each sample reached 6.52 Gb or more, and the percentage of Q30 bases was greater than 94.5% (Supplementary Table S2). The analysis of these datasets revealed that, under control and salt-alkali stress conditions, 8097 genes were differentially expressed in the leaves of ‘D1604’ (4296 genes were upregulated, and 3801 genes were downregulated), while 2545 genes were differentially expressed in the leaves of ‘D1909’ (1449 genes were upregulated, and 1096 genes were downregulated) (Figure 2A). There was a significant difference at the molecular level between the salt-alkali-tolerant cultivar ‘D1909’ (SAT vs. SAT CK) and the salt-alkali-sensitive cultivar ‘D1604’ (NAST vs. NSAT CK), with a total of 1403 DEGs coexpressed in the two samples (Figure 2B). After enrichment analysis of the 1403 DEGs in the KEGG database, significant enrichment of cellular components and molecular functions was detected. KEGG (Figure 2C) and GO enrichment analyses (Supplementary Figure S1 helped to further elucidate the molecular interactions between DEGs. Molecular function screening was performed on the DEGs (Figure 2D), which were mainly enriched in pathways such as terpenoid synthesis, ABC transport, and phenylpropanoid synthesis. Therefore, we focused on 14 genes related to ABC activity that were enriched in the leaves. These genes were differentially expressed in the differentially tolerant lines ‘D1604’ and ‘D1909’ under salt-alkali stress, as shown in Supplementary Table S4.

Among these genes, LOC101216705, LOC101209536, LOC101209574, and LOC101210136 were upregulated in D1909 and downregulated in D1604.

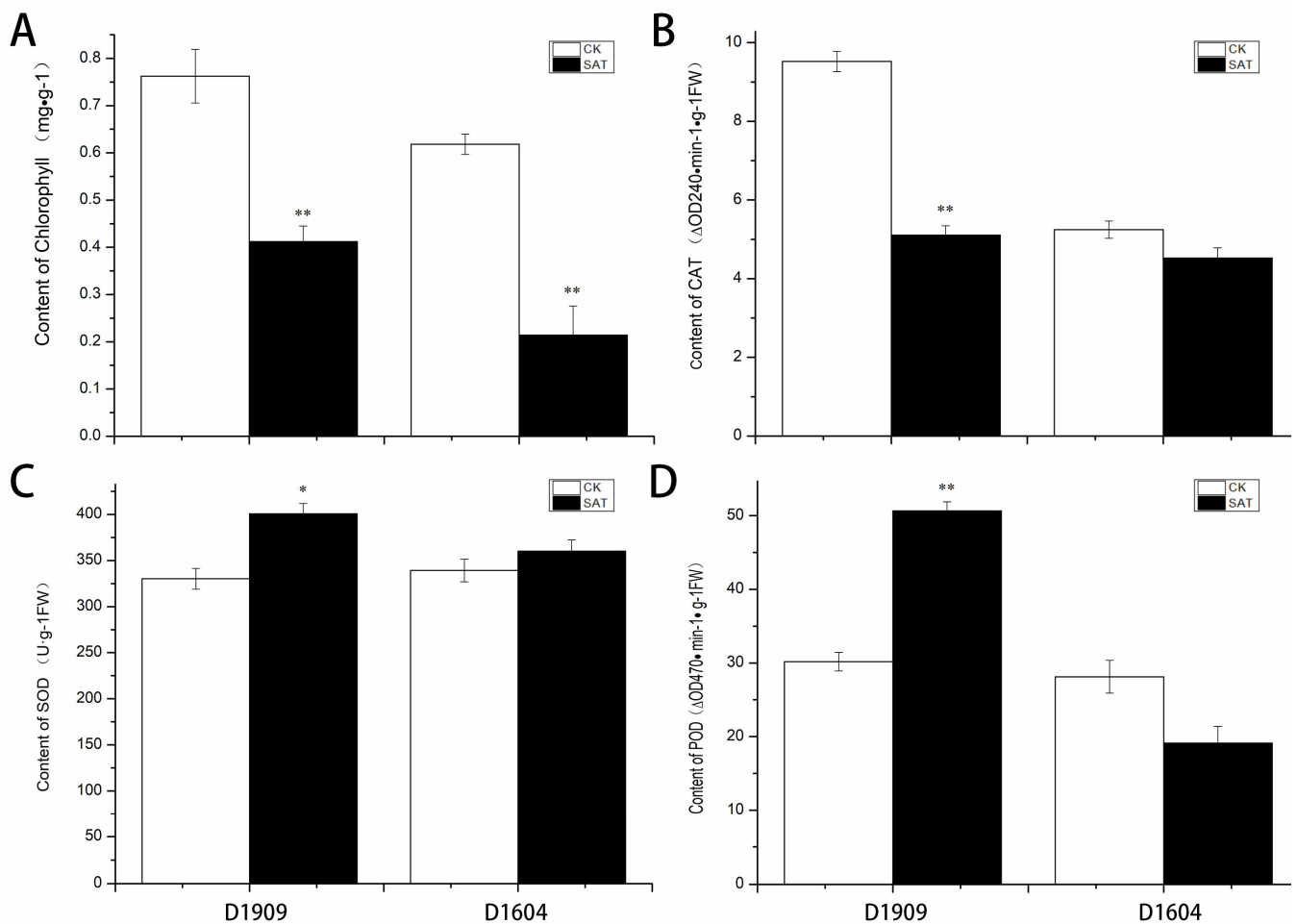


Figure 1. Physiological and biochemical indicators; (A) chloroplasts; (B) catalase; (C) superoxide dismutase; (D) peroxidase; the “*” shows that the value is significant at the 0.05 level based on the Student *t* test; the “**” shows that the value is extremely significant at the 0.01 level based on the Student *t* test.

In addition, the KEGG enrichment results showed that the DEGs in ‘D1604’ were mainly enriched in functional pathways such as linolenic acid metabolism, ABC transport, and DNA replication; the DEGs in ‘D1909’ were mainly enriched in various metabolic pathways, such as arginine biosynthesis, ABC transport, pyrimidine metabolism, and phenylalanine metabolism (Figure 3A,B), indicating that the occurrence of salt–alkali tolerance may be related to multiple metabolic pathways.

To confirm the accuracy of the RNA-Seq results, we randomly selected 15 DEGs (LOC101216705, LOC101209536, LOC101209574, LOC101210136, LOC101207100, LOC101221489, LOC101213089, LOC101217846, LOC105435915, LOC101208704, LOC101204533, LOC116402258, LOC101209789, LOC101213038, and LOC101204949) for qRT-PCR detection. The expression trend of the selected DEGs was consistent with the Illumina sequencing data (Figure 4), indicating the reliability of the RNA-Seq data.

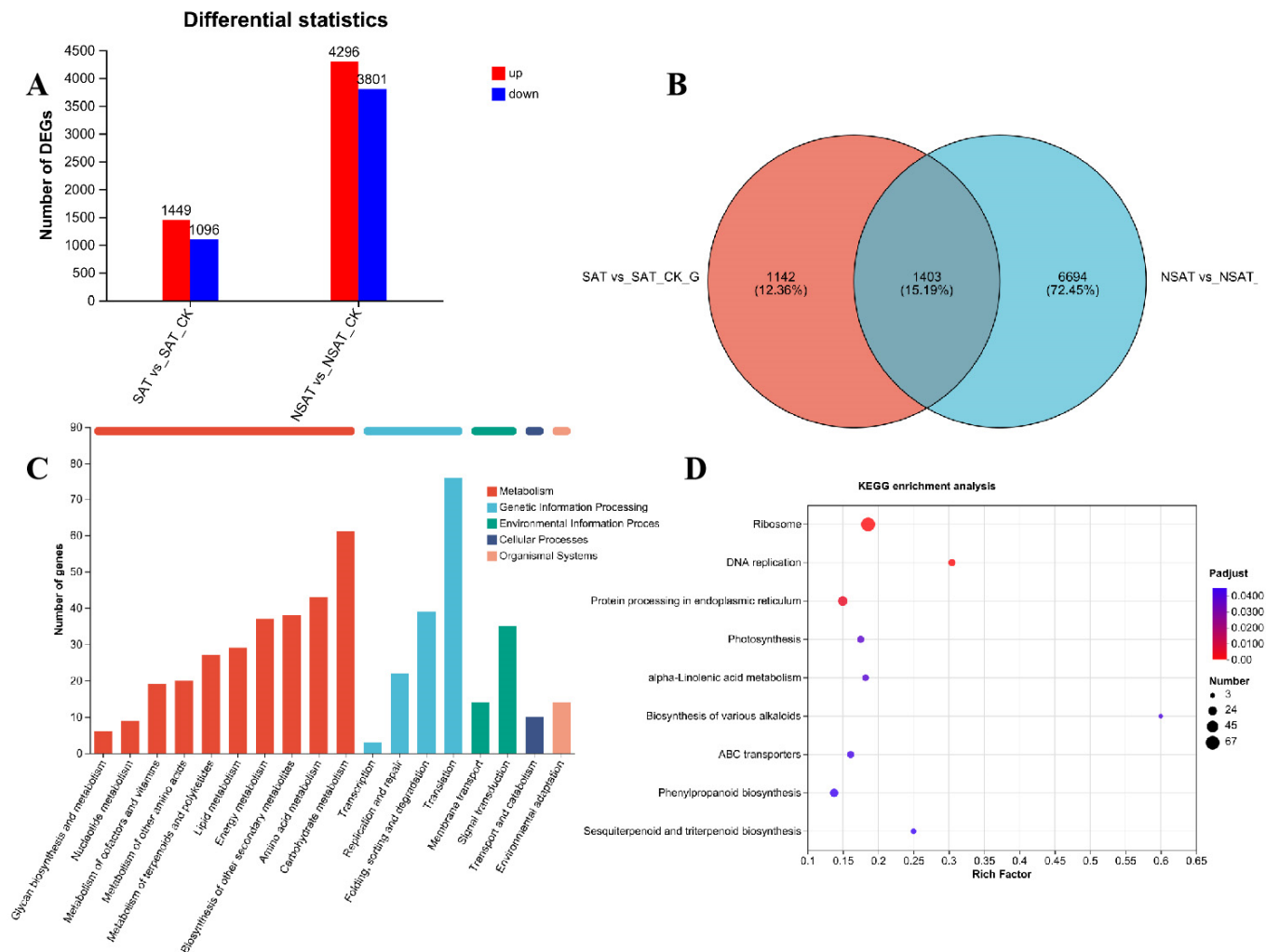


Figure 2. Summary of DEGs between salt-alkali and control. (A) Number of DEGs in different comparisons. SAT: ‘D1909’ salt-alkali treatment; SAT-CK: ‘D1909’ control; NSAT: ‘D1604’ salt-alkali treatment; NSAT-CK: ‘D1604’ control; (B) using the Venn method to analyze the DEG combination (SAT vs. SAT-CK and NSAT vs. NSAT-CK) ($\log_2$ -fold change ≥ 1.5 and FDR-corrected p -value ≤ 0.001); (C) functional annotation of common differentially expressed gene KEGG pathway; (D) common differentially expressed gene KEGG functional enrichment pathway (padjust < 0.05).

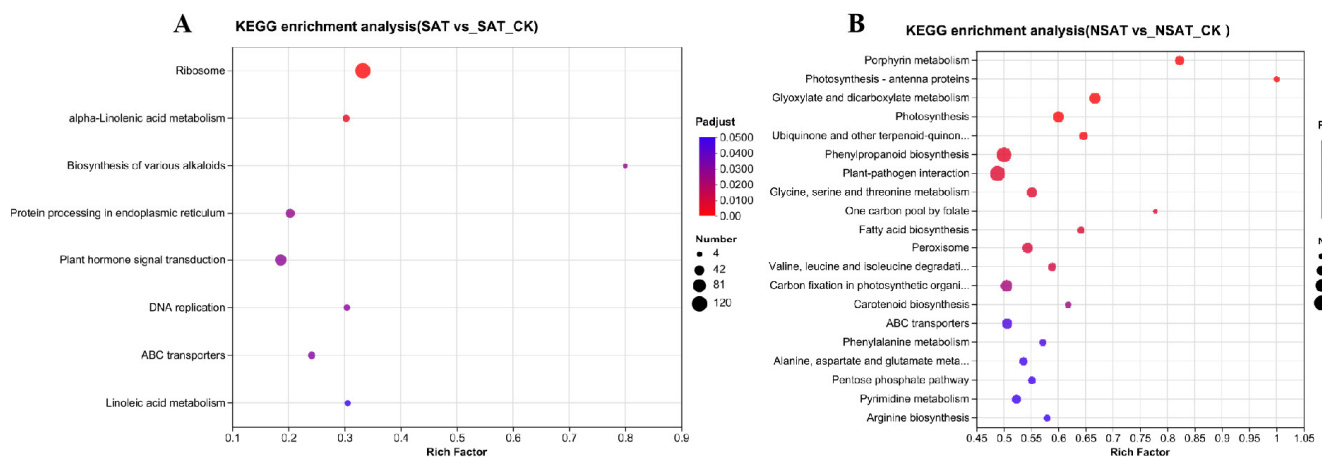


Figure 3. Differential gene enrichment pathways among different cucumber strains. (A) ‘D1909’; (B) ‘D1604’ (padjust < 0.05).

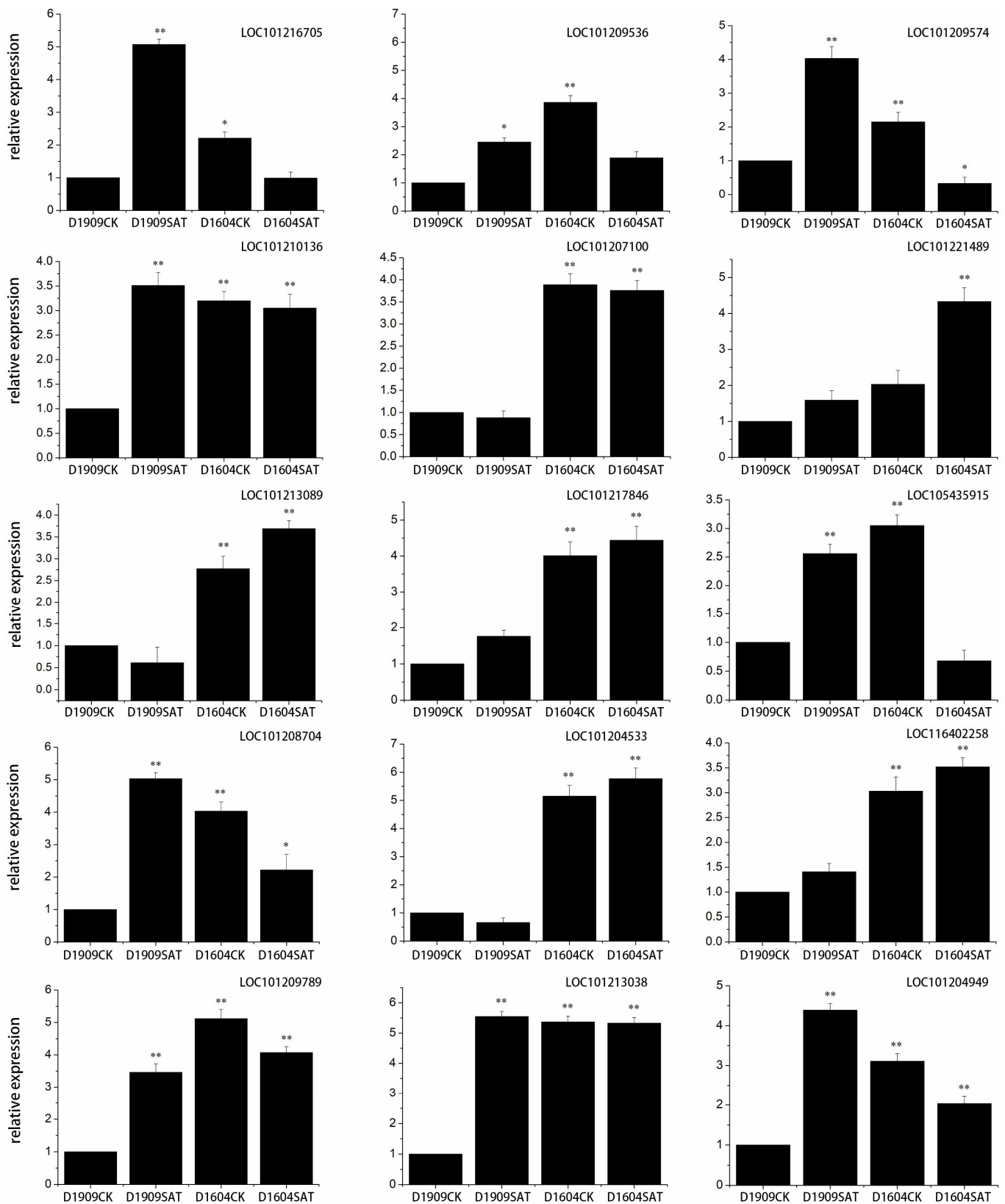


Figure 4. DEGs fluorescence quantification; the “*” shows that the value is significant at the 0.05 level based on the Student *t* test; the “**” shows that the value is extremely significant at the 0.01 level based on the Student *t* test.

3.3. Metabolomic Analysis of ‘D1909’ and ‘D1604’

To further investigate the mechanism of cucumber tolerance to salt–alkali stress, differentially expressed metabolites (DEMs) in cucumber leaves were identified using high-performance liquid chromatography. A total of 90 DEMs were identified through paired sample comparison via widely targeted metabolomics. Fifty-five DEMs were identified in ‘D1604’ (DNSAT vs. DNSAT CK). Of these, 18 metabolites were upregulated, and 37 metabolites were downregulated (Figure 5A). A total of 60 DEMs were identified in ‘D1909’ (DSAT vs. DSAT CK), of which 35 metabolites were upregulated and 25 metabolites were downregulated (Figure 5B). There was a significant difference in metabolite levels between the salt–alkali-tolerant cultivar ‘D1909’ (DSAT vs. DSAT CK) and the salt–alkali-sensitive cultivar ‘D1604’ (DNSAT vs. DNSAT CK). A total of 25 DEMs were coexpressed in the two samples (Figure 5C). Based on the KEGG database, functional enrichment of these 25 DEMs helped us further understand the molecular interactions between DEMs (Figure 5D), which were mainly enriched in pathways such as arginine and proline metabolism, galactose metabolism, glyceride metabolism, and ABC transport.

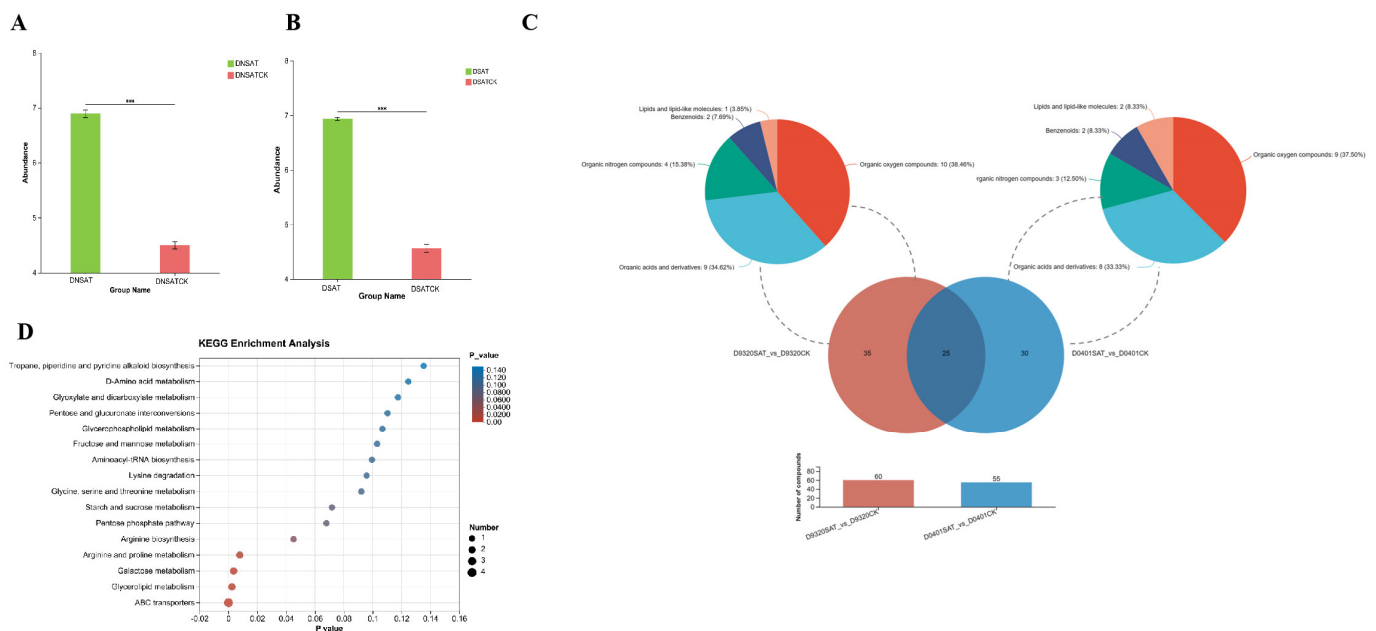


Figure 5. Differential metabolites respond to salt–alkali stress. (A) Bar chart of relative expression abundance of metabolites in salt–alkali sensitivity strain ‘D1604’; (B) bar chart of relative expression abundance of metabolites in salt–alkali tolerance strain ‘D1909’; (C) using the Venn method to analyze the DEMs combination (DSAT vs. DSAT-CK and DNSAT vs. DNSAT-CK) (log₂-fold change ≥ 1.5 and FDR-corrected p -value ≤ 0.001); (D) enrichment of common differential metabolite KEGG pathway (padjust < 0.05); the “****” shows that $p < 0.001$.

In addition, the KEGG enrichment results showed that the DEMs in ‘D1604’ and ‘D1909’ were mainly enriched in metabolic pathways such as ABC transport, sugar metabolism, and amino acid metabolism (Figure 6A,B), but the expression trends were different. The ABC transport pathway exhibited a decreasing trend in both lines, but the degree of downregulation differed. The metabolic pathways associated with amino acids such as propionic acid, alanine, aspartic acid, and arginine exhibited a downward trend in the salt–alkali-sensitive cultivar ‘D1604’. Similarly, the salt–alkali tolerance of cultivar ‘D1909’ tended to increase (Figure 6C,D), indicating that the occurrence of salt–alkali tolerance under salt–alkali stress may be related to multiple metabolic pathways, which is consistent with the transcriptomic results.

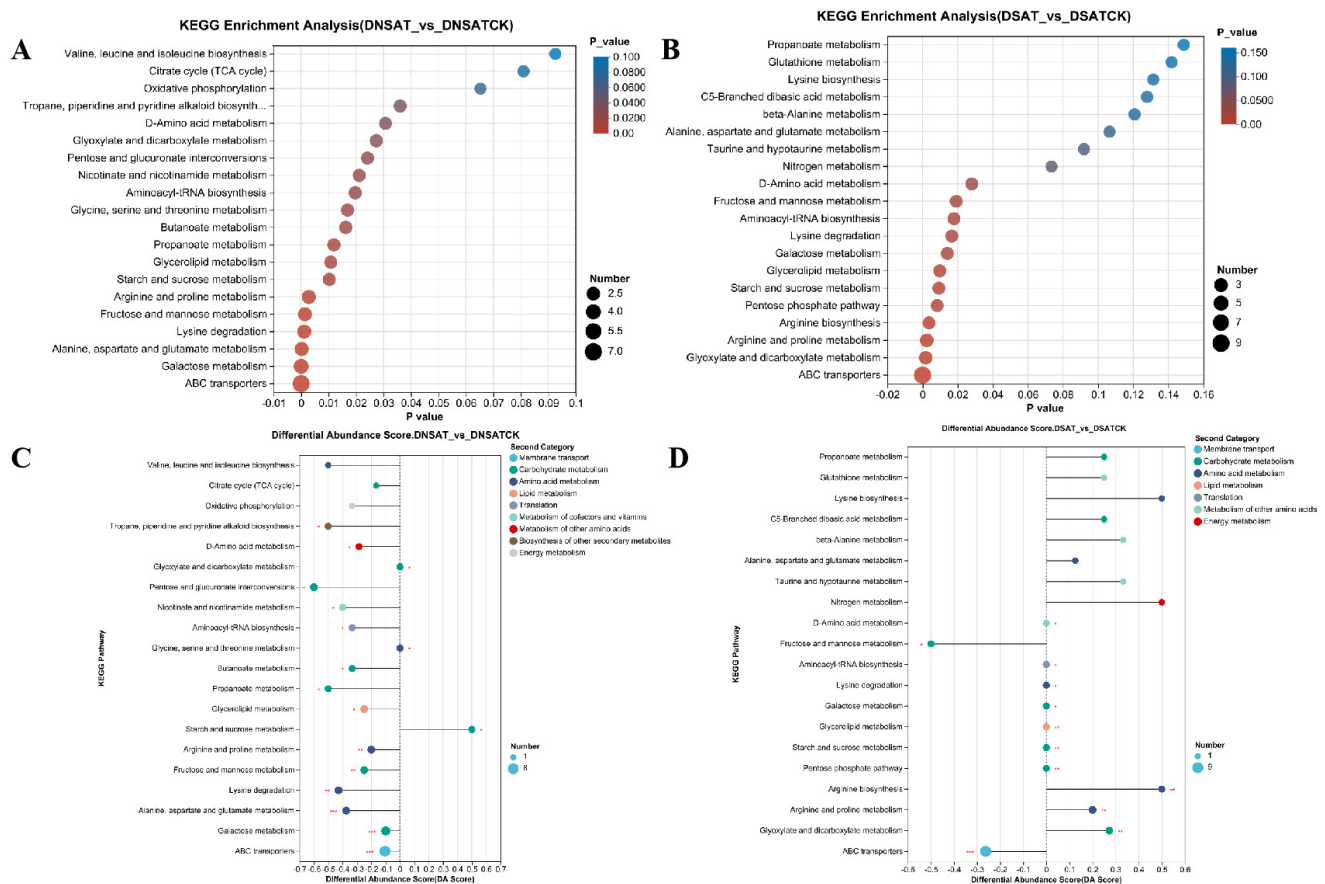


Figure 6. DEMs situation among different cucumber strains. (A) DEMs enrichment pathway in salt-alkali sensitivity strain 'D1604'; (B) DEMs enrichment pathway in salt-alkali tolerance strain 'D1909'; (C) difference abundance score of metabolite KEGG pathway in salt-alkali-resistant strain 'D1604'; (D) difference abundance score of metabolite KEGG pathway in salt-alkali-resistant strain 'D1909'.

3.4. Joint Analysis of Metabolomics and Transcriptomics Data

To better understand the relationships between DEGs and DEMs, metabolomics and transcriptomics data were integrated and analyzed. The association analysis of the KEGG pathway data revealed that ABC transport, galactose metabolism, and starch and sucrose metabolism, as well as metabolic pathways, such as alanine and glutathione metabolism, were activated in both 'D1604' and 'D1909' (Figure 7A,B). A transcriptional regulation pattern diagram was drawn based on the joint analysis of transcription and metabolism (Figure 8).

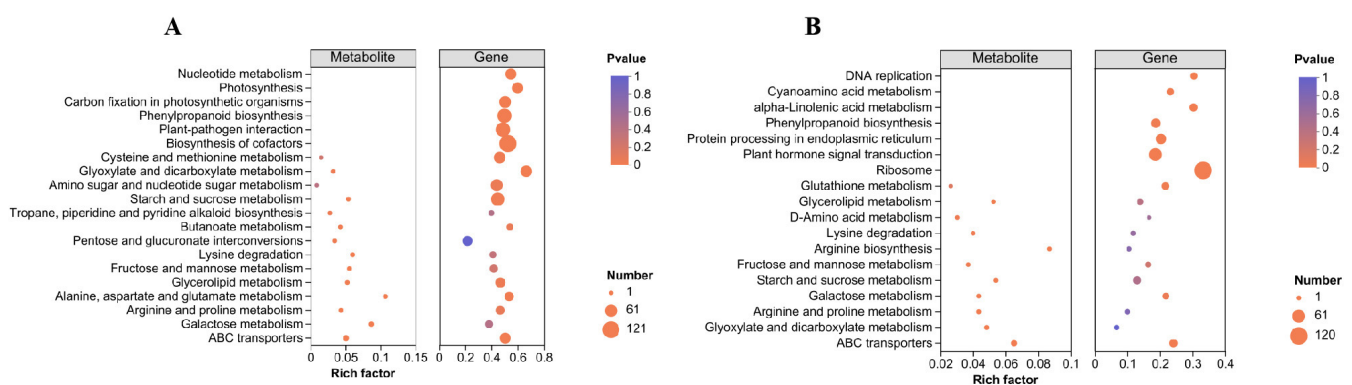


Figure 7. KEGG pathways enriched in integration analysis of metabolomics and transcriptomic data. (A) 'D1604'; (B) 'D1909'.

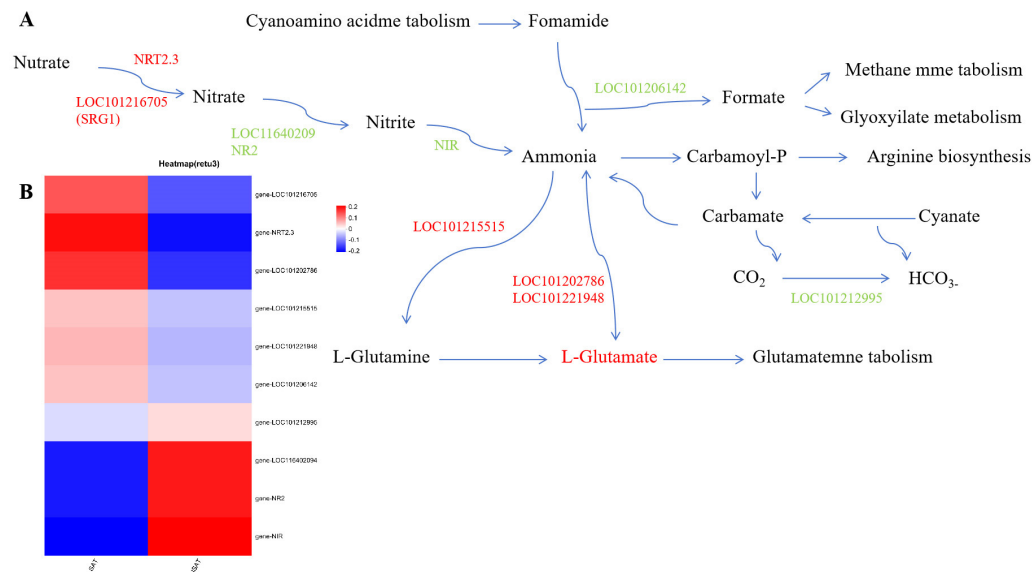


Figure 8. Conjoint Analysis pattern diagram. (A) Metabolic pathway diagram; (B) Heatmap of genes related to metabolic pathways in SAT/NSAT samples.

3.5. Cloning and Sequence Analysis of CsSRG1
3.5.1. Cloning of CsSRG1

Using CsSRG1-F and CsSRG1-R as primers and ‘D1909’ leaf cDNA as a template, we amplified a band of approximately 1200 bp via PCR (Figure 9A). The recovery and purification of the target fragment were performed, and the product was cloned and inserted into the pEASYT3 vector. Sequencing the bacterial culture with the correct band position yielded a complete open reading frame with a sequence of 1152 bp, with a starting codon of ATG and ending codon of TAA, encoding a total of 384 amino acids (Figure 9B). After sequencing, the base sequence was shown to be identical to that of the gene (LOC101216705) in the NCBI database, and the alignment was completely consistent, indicating the successful cloning of CsSRG1.

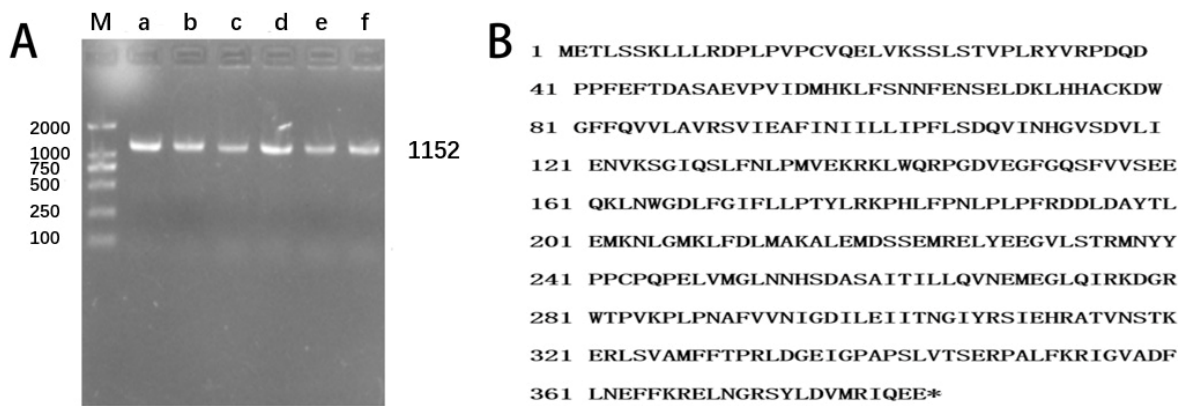


Figure 9. Cloning of CDS sequences and corresponding amino acid sequence of CsSRG1. (A) Gel electrophoresis of PCR products; (B) corresponding amino acid sequence of CsSRG1; M: DNA marker 2K; a, b, c, d, e, f: PCR product. The percentage of the gel was 1%.

3.5.2. Expression Patterns of CsSRG1 in Response to Salt–Alkali Stress

The expression patterns of CsSRG1 in ‘D1909’ and ‘D1604’ were analyzed via qRT-PCR under salt–alkali stress (Figure 10). In ‘D1909’, the expression of CsSRG1 was highly significantly upregulated in tissues other than the roots, and the expression in the leaves was the highest (11.28-fold that of the control group) (Figure 10A). In ‘D1604’, the expression

of *CsSRG1* was highly significantly downregulated in the leaves and stems and significantly downregulated in the fruits, but the difference was not significant in the roots compared to that in the wild-type variant. (Figure 10B). In summary, under salt-alkali stress, the expression pattern of *CsSRG1* differed between ‘D1909’ and ‘D1604’.

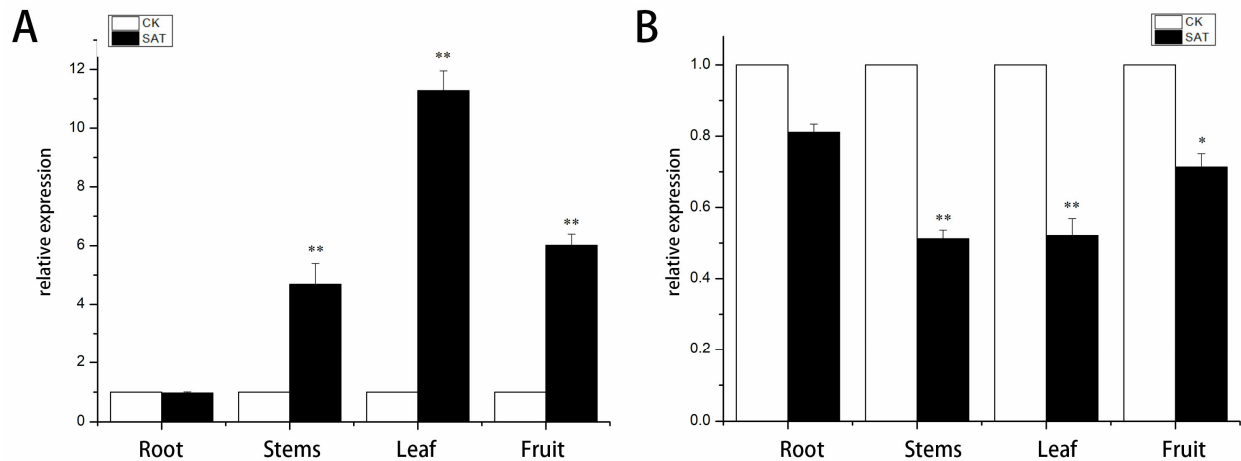


Figure 10. Expression pattern of *CsSRG1* response to saline-alkali stress. (A) Expression pattern of *CsSRG1* in different tissues and organs in ‘D1909’; (B) expression pattern of *CsSRG1* in different tissues and organs in ‘D1604’; the “**” shows that the value is significant at the 0.05 level based on the Student *t* test; the “***” shows that the value is extremely significant at the 0.01 level based on the Student *t* test.

3.5.3. Construction of the *CsSRG1* Expression Vector and Genetic Transformation of Cucumber

We generated the overexpression vectors *CsSRG1* (+)-PCXSN and *CsSRG1* (–)-PCXSN under the control of the strong constitutive CaMV35S promoter. The overexpression vectors *CsSRG1* (+)-PCXSN and *CsSRG1* (–)-PCXSN were successfully transformed into ‘D1909’ and ‘D1604’, respectively, using cucumber genetic transformation technology (Figure 11).

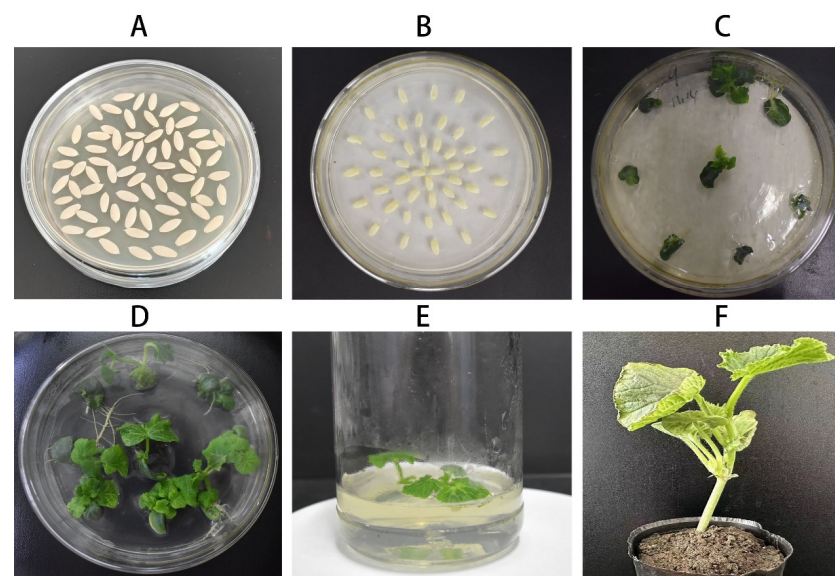


Figure 11. Transformation of cucumber; (A) cucumber seed; (B) co-culture; (C) screening culture; (D) plant regeneration; (E) rooting culture of resistant seedlings; (F) regeneration of resistant seedlings.

DNA was extracted from the leaves of the transgenic plants as a template, and primers were designed based on the pCXSN vector for use in PCR amplification. The pCXSN-

CsSRG1 (+) plasmid was used as a positive control, and water was used as a negative control. The results indicated that the target fragments were approximately 1300 bp in length in the positive control and in some resistant plants, while no target bands were found in the negative control, indicating that the pCXSN-CsSRG1 plasmid was integrated into the cucumber genome (Figure 12A,B).

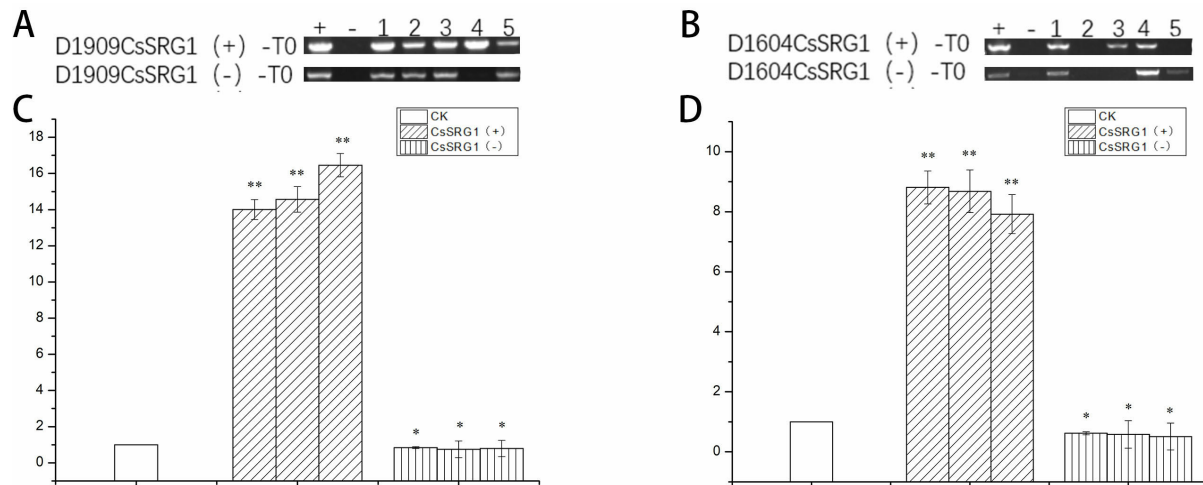


Figure 12. Molecular biological verification of transgenic plants. (A) CsSRG1'D1909'-overexpressing plants T₀ were identified via PCR; (B) CsSRG1'D1604'-overexpressing plants T₀ were identified via PCR; (C) CsSRG1'D1909'-overexpressing plants T₀ were identified via qRT-PCR; (D) CsSRG1'D1604'-overexpressing plants T₀ were identified via qRT-PCR; the “*” shows that the value is significant at the 0.05 level based on the Student *t* test; the “**” shows that the value is extremely significant at the 0.01 level based on the Student *t* test.

To eliminate false positives in resistant plants and to ensure the integrity and accuracy of the experiment, we extracted RNA from the leaves of the transgenic-positive plants and nontransgenic plants and performed reverse transcription to convert this template into cDNA for qRT-PCR identification. The results showed that in 'D1909', CsSRG1 expression significantly increased after the transfection of CsSRG1 (+) (OE1, OE11 and OE15) and was approximately 15.01-fold greater than that in the wild type. After the transfection of CsSRG1 (-) (AS1, AS3 and AS4), the expression of CsSRG1 decreased and was approximately 0.79-fold greater than that in the wild type (Figure 12C). In 'D1604', CsSRG1 expression was upregulated after CsSRG1 (+) transfer (OE6, OE10, and OE17), with an expression level approximately 8.47-fold greater than that in the wild type. After being transferred to CsSRG1 (-) (AS5, AS8, and AS12), the expression of CsSRG1 was approximately 0.57-fold greater than that in the wild type (Figure 12D).

3.5.4. Analysis of Salt-Alkali Tolerance in CsSRG1-Overexpressing Plants

Salt-alkali treatment was applied to the T₀ generation-related CsSRG1-overexpressing plants, as shown in Figure 13. Salt-alkali stress resulted in stunted cucumber plants, wrinkled leaves, and local or overall yellowing and wilting. Among the plants, the ability of 'D1909' and 'D1604' to resist salt-alkali stress was significantly greater than that of the other treatments after the transfection of CsSRG1 (+), while the transfection of CsSRG1 (-) resulted in more severe effects of salt-alkali stress, indicating that CsSRG1 may be a key gene involved in cucumber resistance to salt-alkali stress.

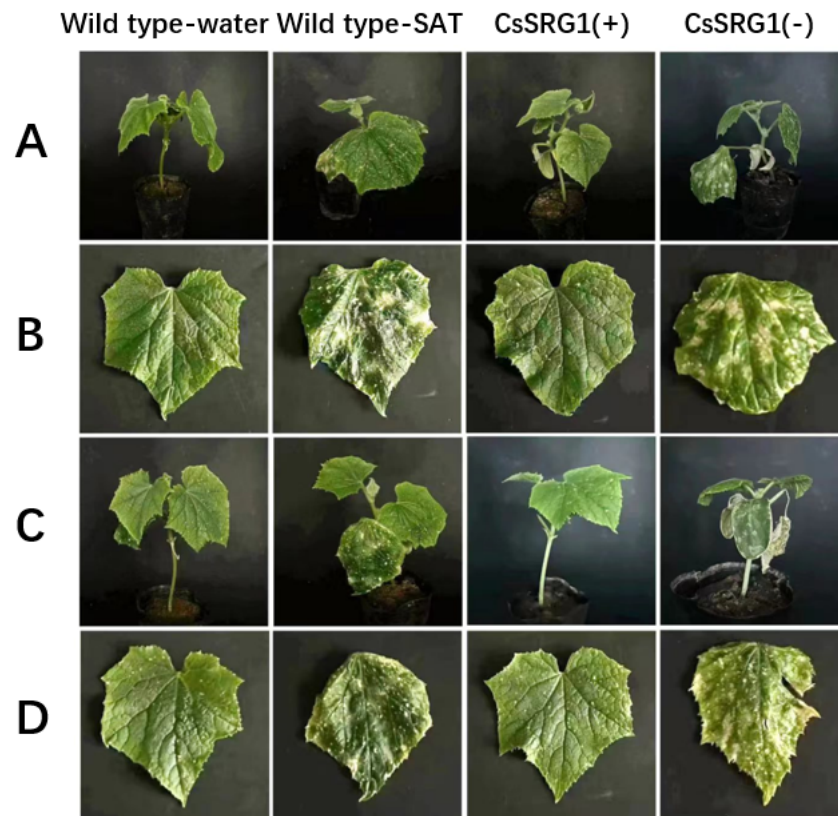


Figure 13. Analysis of salt and alkaline tolerance in *CsSRG1* transgenic-positive plants. (A) cucumber seedlings of 'D1909'-T₀; (B) cucumber leaves of 'D1909'-T₀; (C) cucumber seedlings of 'D1604'-T₀; (D) cucumber leaves of 'D1604'-T₀.

3.5.5. Expression of Key Genes in the ABC Pathway in Overexpression Plants

To investigate whether there is a synergistic effect between other key genes in the ABC pathway and *CsSRG1*, qRT-PCR was performed on the expression of LOC101209536, LOC101209574, and LOC101210136 in overexpressing cucumber plants. Among these proteins, LOC101209536 is a multidrug-resistant protein, LOC101209574 is an ABC transporter B family member, and LOC101210136 is an ABC transporter C family member. As shown in Figure 14, under salt–alkali stress, the expression of LOC101209536 was significantly greater in both D1909 and D1604 than in the control after the transfection of *CsSRG1*. The opposite trend was observed for *CsSRG1* (–), for which there was no significant difference in gene expression compared to that in the control (Figure 14A). Similarly, the expression of LOC101209574 and LOC101210136 was significantly upregulated in both the *CsSRG1* (+) and *CsSRG1* (–) overexpression plants of 'D1909' and significantly downregulated in both the *CsSRG1* (+) and *CsSRG1* (–) overexpression plants of 'D1604' (Figure 14C,E). In the absence of salt–alkali stress, the expression of LOC101209536 was significantly upregulated in 'D1909' and 'D1604' after the transfection of *CsSRG1* (+) compared to that in the control. The expression of the *CsSRG1* (–) gene in the overexpression plants was not significantly different from that in the control plants (Figure 14B), but the expression patterns of LOC101209574 and LOC101210136 were unique. In the 'D1909' and 'D1604' overexpression and nonsensitive plants, the gene expression changed very little compared to that in the control, especially for LOC101209574, whose expression level was almost identical to that in the control (Figure 14D,F).

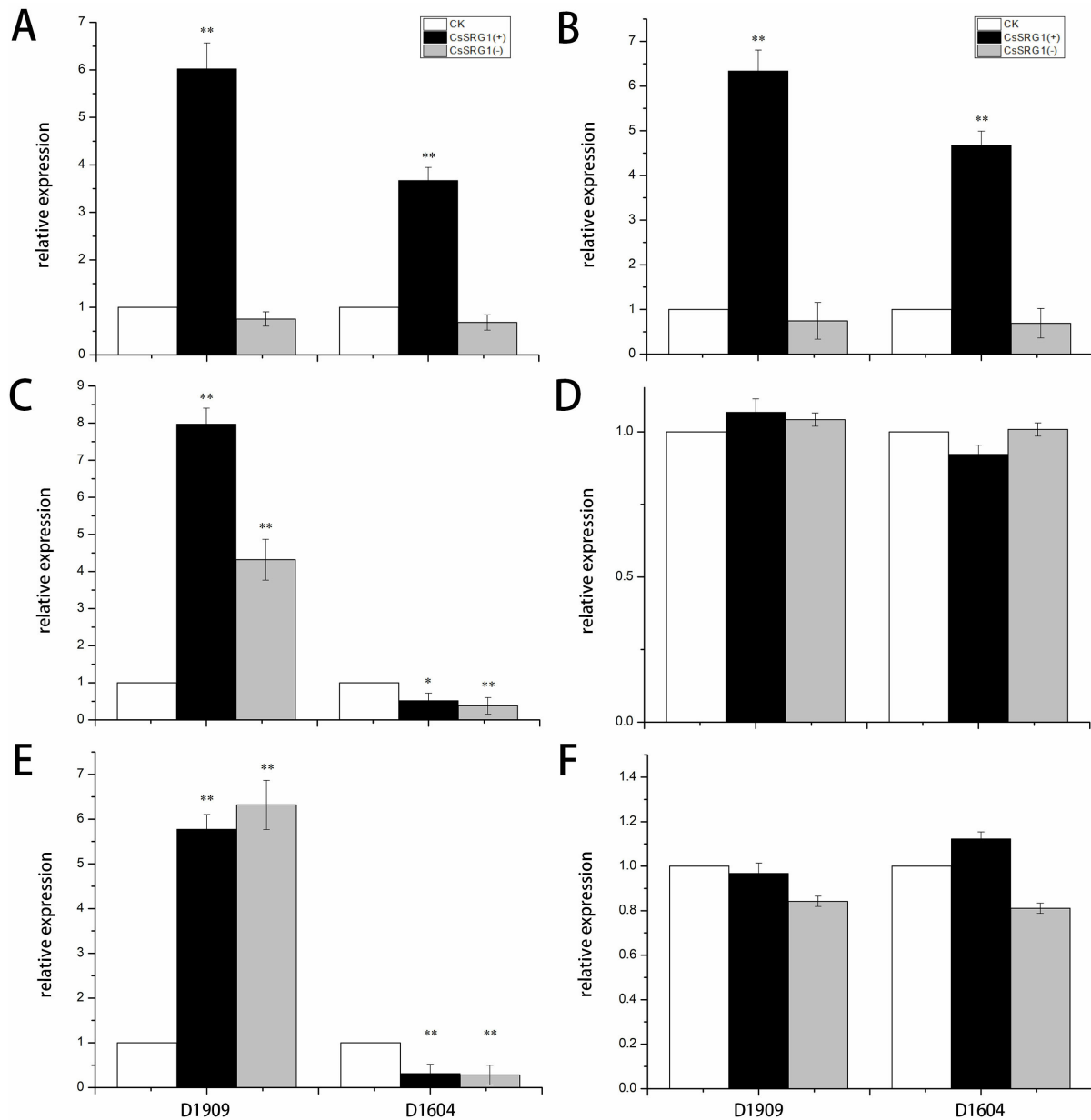


Figure 14. Expression of key genes in positive plants; (A) relative expression of LOC101209536 in ‘D1909’ and ‘D1604’ *CsSRG1* overexpression under salt–alkali stress; (B) relative expression of LOC101209536 in ‘D1909’ and ‘D1604’ *CsSRG1* overexpression under non-salt–alkali stress; (C) relative expression of LOC101209574 in ‘D1909’ and ‘D1604’ *CsSRG1* overexpression under salt–alkali stress; (D) relative expression of LOC101209574 in ‘D1909’ and ‘D1604’ *CsSRG1* overexpression under non-salt–alkali stress; (E) relative expression of LOC101210136 in ‘D1909’ and ‘D1604’ *CsSRG1* overexpression under salt–alkali stress; (F) relative expression of LOC101210136 in ‘D1909’ and ‘D1604’ *CsSRG1* overexpression under non-salt–alkali stress; the “*” shows that the value is significant at the 0.05 level based on the Student *t* test; the “***” shows that the value is extremely significant at the 0.01 level based on the Student *t* test.

4. Discussion

Stress can affect growth and energy metabolism in cucumber plants, and morphogenesis can intuitively reflect the stress status of plants. The impact of salt–alkali stress on cucumber plants occurs throughout the growth cycle, with the response occurring during the seedling stage being the most sensitive. Cucumber seedling quality can be studied by accurately measuring changes in several morphological indicators (plant height, stem

diameter, leaf area, fresh mass, and dry mass) and physiological and biochemical indicators. Under salt–alkali stress, the dry mass of cucumber plants significantly decreases, and growth indicators such as stem diameter, plant height, number of leaves, and leaf area severely decrease [20]. In the present study, under salt–alkali stress, there were certain differences in various indicators among the 32 cultivars. The morphological indicators of ‘D1909’ were significantly greater than those of the other 31 varieties, while ‘D1604’ had a slightly greater stem diameter than D1104-2-4 did, and all the other indicators were lower than those of the other cultivars. ‘D1604’ withered and had large areas of yellow spots on its leaves; these areas were more severely affected by salt–alkali stress than D1909. An excessive salt content in soil can reduce plant photosynthesis, thereby inhibiting the accumulation of photosynthetic products in crop leaves and inhibiting plant growth and development. In severe cases, this can lead to crop death [21,22]. Under stress conditions, salinity can stimulate an increase in the content of protective enzymes in the plant’s inner membrane system, scavenge oxygen free radicals in the body, and reduce damage to the plant [23]. The chlorophyll content and membrane-protective enzyme content of ‘D1909’ were greater than those of ‘D1604’ under salt–alkali stress in the present study, which was consistent with previous results. In summary, under salt–alkali stress, there were significant differences in morphological and physiological indicators between ‘D1909’ and ‘D1604’. ‘D1909’ had a significantly greater tolerance to salt–alkali conditions than D1604. Therefore, ‘D1909’ was chosen as the salt–alkali-tolerant cultivar, and ‘D1604’ was chosen as the salt–alkali-sensitive cultivar for subsequent experiments.

As soil salinization becomes increasingly severe, it is particularly important to study the transcriptional and metabolic regulatory networks of cucumber plants under salt–alkali stress. This study involved transcriptional metabolic analysis of salt–alkali-tolerant and salt–alkali-sensitive cucumber cultivars in order to determine the mechanism of salt–alkali tolerance in these plants. During transcriptome analysis, a total of 1403 coexpressed DEGs were identified between ‘D1909’ (SAT vs. SAT-CK) and ‘D1604’ (NST vs. NST-CK). Their functions were mainly enriched in pathways such as terpenoid synthesis, ABC transport, and phenylpropanoid synthesis. Terpene compounds are important secondary metabolites, and the accumulation of secondary metabolites not only allows plants to adapt to their living environment but also enhances their own defense mechanisms [24]. In tobacco research, it was found that terpenes accumulated under salt–alkali stress. ABC transporters can participate in the body’s tissue defense programs, including the absorption of various toxic substances, metabolite accumulation, and toxin metabolism and excretion processes [25]. One of the end products of the phenylpropanoid biosynthesis pathway is lignin, which is the main structural component of plant cell walls and can increase the mechanical strength to allow plant cells to resist damage caused by stress [26]. William et al. reported that the tolerance of barley to salt–alkali stress is related to the levels of phenylpropanoids and suberin in the roots [27].

During the metabolomic analysis, 25 DEMs were found to be coexpressed in both the salt–alkali-tolerant cultivar ‘D1909’ (DSAT vs. DSATCK) and the salt–alkali-sensitive cultivar ‘D1604’ (DNSAT vs. DNSATCK). The DEMs were mainly enriched in pathways related to arginine and proline metabolism, galactose metabolism, glyceride metabolism, and ABC transport. Under salt–alkali stress, plants restructure their carbon and nitrogen metabolism balance, leading to a shift from carbon metabolism toward nitrogen metabolism and to the increased accumulation of nitrogen-containing organic compounds, such as proline and free amino acids, thus enhancing osmotic regulation and salt–alkali adaptability [28,29]. Research has shown that the accumulation of amino acid metabolites in plants is closely related to salt tolerance. Importantly, amino acids accumulate significantly in plant leaves and participate in osmotic regulation, reactive oxygen species scavenging, and perform ion transport, alleviating the physiological damage caused to mesophyll cells by salt–alkali stress [30,31].

Joint analysis revealed significantly differentially abundant pathways involved in ABC transport at both the transcriptomic and metabolomic levels, and the functional

differences in plant ABC transport proteins were significant, with diverse and complex structures. The ABCB subfamily can increase tolerance to abiotic stress, and most of the *SlABCBs* in tomatoes are involved in ion and heavy metal transport in the roots [32]. ABCC participates in the transport of secondary metabolites and detoxifies heavy metals. Arabidopsis *AtABCC1* and *AtABCC2* have a certain tolerance to arsenic, cadmium, and mercury [33]. The ABCG subfamily mainly participates in the transport of metabolites and hormones and plays an important role in the secretion of secondary metabolites [34]. These proteins can confer resistance to various stress conditions, allow plants to respond to pathogens and microorganisms, regulate heavy metal balance, transport plant secondary metabolites, and regulate plant growth and development. It follows that the study of ABC transporters is highly important for understanding the mechanism of cucumber response to salt-alkali stress. The *SRG1* gene is located at a critical position in the pathway and participates in the transformation of nitrate into nitrite. This gene was upregulated in the salt-alkali-tolerant cultivar 'D1909' and downregulated in the salt-alkali-sensitive cultivar 'D1604'. It is speculated that *SRG1* may be a key gene in cucumber 'D1909' that can confer resistance to salt-alkali stress. Therefore, this gene was selected for functional verification and named *CsSRG1*.

Analyzing the expression patterns of genes is important for the elucidation of gene functions. We found that *CsSRG1* was constitutively expressed and exhibited obvious intertissue differences, particularly in the leaves, stems, and fruits. The expression levels in different tissues and organs were ordered as follows: leaves > stems > fruit > roots. The expression level of *CsSRG1* in the roots, stems, leaves, and fruits of 'D1909' was significantly greater than that in the same organs of 'D1604'. Research on a gene related to the ABC family, *CsABC19*, in cucumber showed that *CsABC19* is expressed mainly in cucumber leaves [35]. In the present study, through tissue-specific analysis, *CsSRG1*, a transcription factor that can regulate the expression of cucumber ABC family proteins, was shown to regulate the expression of cucumber ABC family proteins and simultaneously participate in the transformation of nitrate into nitrite. *CsSRG1* can actively respond to salt-alkali stress and may be involved in salt-alkali tolerance, participate in salt-alkali degradation, and play an important role in reducing salt-alkali damage to cucumber fruits, stems, and leaves.

To investigate whether there is a synergistic effect between other key genes in the ABC pathway and *CsSRG1*, genes with the same expression pattern as *CsSRG1* in the ABC pathway, namely, LOC101209536, LOC101209574, and LOC101210136, which were upregulated in the salt-alkali-tolerant cultivar 'D1909' and downregulated in the salt-alkali-sensitive cultivar 'D1604', were selected as the research objects. The expression levels of these genes in the overexpression cucumber plants were tested via qRT-PCR. LOC101209536 exhibited the same expression pattern as *CsSRG1* under salt-alkali stress, while LOC101209574 and LOC101210136 were upregulated in 'D1909' cucumber plants and downregulated in 'D1604' cucumber plants overexpressing the gene, which was consistent with the transcriptome sequencing results. However, in the absence of salt-alkali stress, only LOC101209536 exhibited the same expression pattern as *CsSRG1*. LOC101209536 is a multidrug resistance-associated protein. Previous studies have shown that plant PDR proteins can actively respond to various biotic and abiotic stresses; participate in the absorption, accumulation, and emission of toxic substances; play an important role in the transport and accumulation of some secondary metabolites; and improve plant resistance. *OsPDR1* is expressed in various tissues of rice and can effectively enhance its salt tolerance [36]. *AtPDR8* is upregulated under salt stress, and its encoded protein is involved in the response to various biotic and abiotic stresses [37]. It can be inferred that the overexpression of *CsSRG1* promotes an increase in the expression of LOC101209536. As a PDR protein, LOC101209536 can effectively respond to salt-alkali stress and improve cucumber tolerance to salt-alkali conditions. LOC101209536 and *CsSRG1* have a certain synergistic effect on plant salt-alkali tolerance. In the 'D1909' and 'D1604' cucumber plants overexpressing the gene, the LOC101209574 and LOC101210136 levels were not significantly different from those in the control group in the absence of salt-alkali stress, indicating that

their expression was not affected by *CsSRG1*. Although they can respond to salt–alkali stress and improve plant salt–alkali tolerance, they have independent mechanisms of action and are not related to *CsSRG1*.

5. Conclusions

This study screened the salt–alkali-tolerant cultivar ‘D1909’ and the salt–alkali-sensitive cultivar ‘D1604’ from 32 different cucumber ecological types on the basis of morphological indicators and salt–alkali indices, combined with relevant physiological and biochemical indices, and demonstrated that there was indeed a significant difference in the performance of ‘D1909’ and ‘D1604’ under salt–alkali stress. Further transcriptomic and metabolomic analysis was subsequently conducted to screen the key gene *CsSRG1* from the cucumber cultivar ‘D1909’, which responds to salt–alkali stress. The function of the gene in the cucumber plants was verified through cloning and genetic transformation, revealing the molecular mechanism of cucumber salt–alkali tolerance. This study provides high-quality seed resources for cucumber salt–alkali tolerance breeding and a theoretical reference to assist in addressing salt–alkali stress-related problems for other plants.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14030543/s1>, Table S1: Primers for differentially expressed genes. Table S2: Data statistics table. Table S3: ABC pathway differentially expressed genes. Table S4: Differential metabolites. Figure S1: common differentially expressed gene GO functional enrichment pathway.

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