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GGE Biplot-Based Transcriptional Analysis of 7 Genes Involved in Steroidal Glycoalkaloid Biosynthesis in Potato (*Solanum tuberosum* L.)

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Abstract: Steroidal glycoalkaloids (SGAs) are secondary metabolites that are closely associated with the sensory and processing qualities of potato tubers. GGE biplots are a widely used tool for analyzing crop breeding analysis. This study aimed to investigate the effect of light on SGA biosynthesis by employing GGE biplots to analyze the transcriptional gene expression of seven genes involved in the SGA biosynthesis pathway. Tubers of five different potato genotypes were incubated for 6, 12, and 24 h under red light. The expression levels of the seven genes were measured using qRT-PCR for analysis. Further analysis of the data was performed using GGE biplots. Our results indicated significantly higher expression levels for *Pvs1*, *Sgt1*, and *Sgt3* genes than those of the remaining tested genes. Across the three red light illumination durations, *Sgt3* showed high and stable expression, although it showed less stability across the different genotypes. Interestingly, the expression patterns of the seven genes were extremely similar for the 12 h and 24 h treatments. It was found that at least 6 h of red light illumination was required for optimal gene expression in all five genotypes, particularly in the genotype Zhuangshu-3 (DXY) after 24 h of treatment. Additionally, significant expression of the seven genes was observed in the L-6 genotype after 12 and 6 h of red light illumination. These results highlight that GGE biplots are an appropriate tool for analyzing and illustrating the differential expression profiles of the seven key genes involved in SGA biosynthesis in potato tubers. This study provides valuable insights into the biosynthesis and metabolism of SGAs in potatoes. Moreover, it demonstrates the potential application of GGE biplots in crop breeding and other research fields.

Keywords: potato; GGE biplot; steroidal glycoalkaloids; gene expression

Citation: Zhao, F.; Li, Y.; Cui, T.; Bai, J. GGE Biplot-Based Transcriptional Analysis of 7 Genes Involved in Steroidal Glycoalkaloid Biosynthesis in Potato (*Solanum tuberosum* L.). *Agronomy* **2023**, *13*, 2127. https://doi.org/10.3390/agronomy13082127

Academic Editors: Mavromatis Theodoros, Thomas Alexandridis and Vassilis Aschonitis

Received: 7 July 2023
Revised: 9 August 2023
Accepted: 9 August 2023
Published: 14 August 2023

1. Introduction

Steroidal glycoalkaloids (SGAs) are secondary metabolites found in a wide range of plant species, including Solanaceae and some economically important crops such as potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum*) [1,2]. Two compounds, i.e., a-solanine and a-chaconine, account for approximately 95% of the total glycoalkaloids (TGA) present in potato cultivars [3]. SGAs, such as solamargine, are the main components in some medicines with anti-inflammatory effects against herpes viruses and cancer cells [4,5]. SGAs present in potatoes may also aid in preventing invasion by microbes and pests [6,7]. The solanine content in potato tubers is influenced by various environmental conditions. Different wavelengths of light have disparate effects on SGA accumulation in potato tubers. Red light (640 nm–680 nm) is the most effective, followed by blue light

(400 nm–450 nm), while the effects of orange, yellow, and green light are not significant. Purple light is generally ineffective for most potato varieties [8].

The biosynthetic pathway of SGAs can be divided into three steps: terpenoids, sterols, and solanidine [9]. As shown in Figure S1, key enzymes such as HMGR (3-hydroxy-3-methylglutaryl coenzyme A reductase 1 & 2), PSS1 (squalene synthase), PVS1 (vetispiradiene sesquiterpene cyclase), SGT1 (solanidine galactosyltransferase), SGT2 (solanidine glucosyltransferase), and SGT3 (rhamnosyltransferase) mainly regulate the process of SGA synthesis [10]. Studies have shown that these enzymes interact with each other during the process of SGA synthesis [11,12]. In addition, several other enzymes are involved in SGA biosynthesis and play important roles in regulating the process. Therefore, a systematic analysis of the expression of key genes involved in the SGA biosynthesis pathway could provide a theoretical basis for controlling and improving the quality of potato tubers using biotechnology in the future.

Yan et al. [13] proposed the GGE biplot analysis method for the graphical representation of genotype—environment (GE) interaction patterns in multi-environment trials (MET). GGE biplots are part of an effective method based on principal component analysis (PCA) for exploring MET data. It combines the biplot concept [14] and the GGE concept [15]. A detailed description of the principles of the GGE biplots can be found in the review by Yan and Tinker [16]. GGE biplots can visualize two-way data types [15] and have been widely used in crop yield and quality assessment [17,18] and genetic analysis [19,20].

In this study, we innovatively used a GGE biplot to analyze the expression of genes involved in steroidal glycoalkaloid biosynthesis in potato microtubers, aiming to reveal the light-mediated regulation of SGA biosynthesis-related gene expression. The results of this study can provide important references for the comprehensive analysis of SGA biosynthesis and metabolism in potato. In addition, the results are expected to be useful for researchers using GGE biplots not only in crop breeding but also in other research fields.

2. Materials and Methods

2.1. Plant Materials and Microtuber Induction

Five potato genotypes were used in the experiments: one was the wild species *S. chacoense* (L-6) with a high level of SGAs [10]; the other four genotypes were cultivated *S. tuberosum*-Shepody (D-3), Favorita (D-6), Longshu-3 (JZ-12), and Zhuangshu-3 (DXY).

Both Longshu-3 and Zhuangshu-3 are indigenous species, with Longshu-3 being light-insensitive and Zhuangshu-3 being light-sensitive. All genotypes were obtained from the Potato Collection Centre of Gansu Key Laboratory of Crop Genetic and Germplasm Enhancement, Gansu Agricultural University.

Flasks (250 mL) were established with eight plantlets that were induced by incubating the cultures for 16 h per day (3000 lx of fluorescent light at 22 $\pm$ 2 °C). Each semi-sealed flask contained 50 mL of semi-solid MS basal medium without agar. The remaining culture fluid was removed from the flask and fresh inductive medium (MS medium, 8% sucrose, 0.15% active carbon) was added to the flask after 4 weeks. Sixty days after incubation in darkness, microtubers were harvested for light treatments, mRNA extraction, and qRT-PCR analysis.

2.2. Light Treatment

The harvested microtubers, with similar diameters of approximately 8 mm, were carefully selected and washed by hand with running water. These microtubers were then subjected to two different experimental conditions. In one condition, the microtubers were entirely wrapped with aluminum foil to create a dark environment. In the other condition, the microtubers were exposed to red light, with an intensity of 10–12 lx, produced by a 15 W red light bulb. Both sets of microtubers were incubated at a temperature of 15 °C. Freshly harvested tubers were used. Following the specified incubation periods of 6, 12, and 24 h, the tubers were treated with liquid nitrogen and immediately stored at −80 °C for subsequent mRNA extraction.

2.3. Total RNA Extraction and cDNA Synthesis

Total RNA extraction was performed on 1 g of frozen microtubers using the Simple Total RNA Kit (Tiangen Biotech Co., Beijing, China) with three replicates according to the manufacturer's instructions. The concentration of total RNA was measured using an Ultrospec 1100 pro spectrophotometer (Amersham Biosciences, Beijing, China). cDNA was synthesized from the total RNA using the PrimeScript® RT reagent kit (TaKaRa Biotechnology Co., Dalian, China) according to the manufacturer's protocol. Before conducting the real-time quantitative polymerase chain reaction (PCR), the synthesized cDNA was diluted with 25 µL of nuclease-free water.

2.4. Real-Time PCR and Data Analysis

The internal reference gene was Actin I (NCBI accession No. XM_015308091.1) [21,22], and relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method [23]. Real-time quantitative PCR was performed using the Mx3005p Multiplex Quantitative PCR System. Primer Premier 5.0 software (Premier Biosoft International) was utilized to design eight pairs of primers, which were based on the potato nucleotide sequences obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/, accessed on 13 February 2022). As shown in Table S1, the standard curve was constructed using standard samples and the designed specific primers. Baseline and threshold values were set in the Mx3005p Multiplex Quantitative PCR System, and the resulting CT values were exported to Microsoft Excel for analysis.

The seven target enzymes involved in the SGA biosynthetic pathway are as follows: hydroxymethylglutaryl coenzyme A reductase (*Hmg1*, *Hmg2*), squalene synthase (*Pss1*), vetispiradiene synthase (*Pvs1*), solanidine galactosyl transferase (*Sgt1*), solanidine glucosyltransferase (*Sgt2*), and rhamnosyltransferase (*Sgt3*).

All data were organized in a two-way table and analyzed using the GGE biplot program (version 7.9) [24]. Data analysis and presentation followed the guidelines described in the review by Yan and Tinker [16] (original data presented in Table 1).

Table 1. ANOVA of potato gene expression in three environments across five genotypes.

Source of Variation		*hmg1*	*hmg2*	*pss1*	*pvs1*	*sgt1*	*sgt2*	*sgt3*
G	D-3	0.44 ± 0.09d	0.50 ± 0.04d	0.69 ± 0.07d	1.64 ± 0.35d	0.46 ± 0.12e	0.85 ± 0.08ab	0.34 ± 0.04c
	D-6	0.36 ± 0.05d	0.84 ± 0.13d	1.52 ± 0.28b	10.45 ± 4.59a	1.57 ± 0.48d	1.02 ± 0.17b	1.30 ± 0.44c
	JZ-12	2.91 ±0.09b	3.23 ± 0.79b	1.46 ± 0.12b	3.15 ± 0.33c	5.92 ± 1.98c	0.78 ± 0.05ab	9.26 ± 1.19b
	DXY	4.59 ± 0.35a	5.75 ± 2.41a	3.20 ±1.19a	7.51 ± 3.39b	8.81 ± 2.76a	4.85 ± 1.96a	13.45 ± 4.03b
	L-6	2.47 ± 0.33c	1.85 ± 0.21c	1.15 ± 0.19c	1.42 ± 0.60d	7.79 ±2.14b	0.59 ± 0.11c	73.35 ± 27.08a
E	6	2.01 ± 0.33b	1.28 ± 0.11b	1.31 ± 0.13b	7.04 ± 2.93a	5.04 ± 1.30a	0.87 ± 0.07b	9.49 ± 2.16b
	12	1.94 ± 0.43b	0.89 ± 0.15c	0.88 ± 0.06c	1.74 ± 0.34c	4.65 ± 1.57a	0.70 ± 0.09b	37.80 ±19.21a
	24	2.50 ± 0.58a	5.13 ± 1.48a	2.62 ±0.74a	5.71 ± 2.08b	5.03 ± 1.95a	3.28 ±1.26a	11.33 ± 2.90b
ANOVA	G	163.034 ***	327.215 ***	116.179 ***	61.644 ***	116.876 ***	472.073 ***	156.045 ***
	E	8.014 **	659.458 ***	175.514 ***	49.263 ***	0.685 ns	499.283 ***	69.789 ***
	G *E	7.365 ***	296.721 ***	150.431 ***	112.907 ***	133.742 ***	429.882 ***	104.185 ***

Note: Values indicate the gene expression mean ± SE (standard error). ns—not significant. Duncan's multiple comparison method was used and different lower-case letters represent significant differences in genotype or environment. * Significant at the 0.05 level of probability. ** Significant at the 0.01 level of probability. *** Significant at the 0.001 level of probability.

3. Results

3.1. The Relevance of Gene Expression and Treatments

The lines connecting the markers for different treatments in the biplots were defined as treatment vectors. The angle between two treatment vectors represents the correlation coefficient between them, with acute angles indicating a positive correlation, obtuse angles indicating a negative correlation, and right angles indicating no correlation [15,25].

The small angle observed between the 12 and 24 h treatments suggests that there is a close similarity in gene expression between these two time points. On the other hand,

the angle between the 6 h treatment and the other two treatments was larger, indicating a greater difference in gene expression between the short- and long-term treatments, although the angle was still less than 90°.

The angles between the JZ-12, L-6, and DXY vectors were less than 90°, indicating a high correlation in the expression of the seven genes between the three genotypes after red light treatment, with a particularly close expression trend observed between L-6 and JZ-12 vectors. Similarly, the angle between the D-3 and D-6 vectors was less than 90°, indicating a high correlation in the expression of the *Sgt3* gene between the two genotypes after red light treatment. On the other hand, the angles between *Hmg2*, *Hmg1*, *Pss1*, and *Sgt2* with different genotypes were all greater than 90°, suggesting a negative correlation between them (Figure 1b).

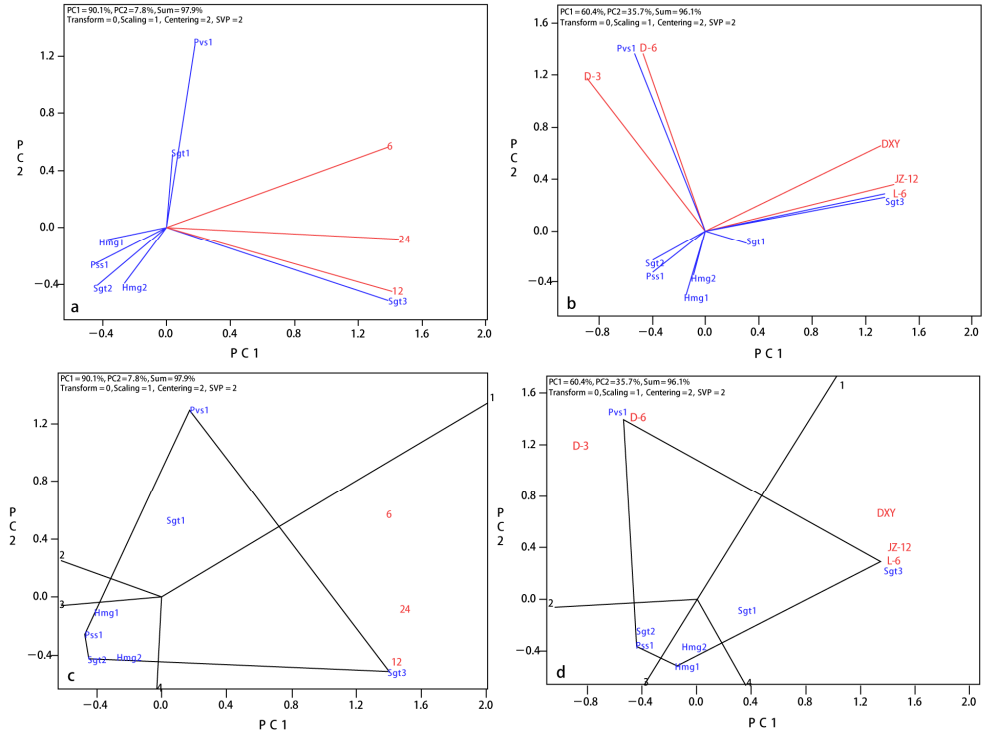

Figure 1. The GGE Biplot analysis showing relevance of gene expression and treatments (red light illumination duration and genotypes) and the polygon view of gene expression—treatment interaction for seven genes in five genotypes under three red light illumination durations. (**a**) Relevance of gene expression and red light illumination duration. (**b**) Relevance of gene expression and genotypes. (**c**) The polygon view of the interaction between gene expression and red light illumination durations interaction. (**d**) The polygon view of the interaction between gene expression and genotypes. The numbers 6, 12, and 24 represent the three illumination durations; D-3, D-6, DXY, JZ-12, and HA represent genotypes involved in the experiment. *Pvs1*, *Sgt1*, *Sgt2*, *Sgt3*, *Pss1*, *Hmg1*, and *Hmg2* represent seven target enzymes involved in the SGA biosynthetic pathway. The plot is based on gene centering (center = 2) with SD scaling and without transformation of data (scaling = 1, transform = 0), and it is gene metric-preserving (SVP = 2).

3.2. Polygon View of Gene Expression—Treatment Interaction

To determine the presence of different genotypes within the regions, a polygon graph was created to visualize the interaction patterns between genotypes and treatments [25]. A polygon was drawn around genotypes that were located away from the biplot origin, encompassing all other genotypes. Perpendicular lines were then drawn from the biplot origin to each side of the polygon, dividing the graph into four sectors (Figure 1). The genotype located at the vertex of each sector, with markers falling into that sector, was considered to be the best genotype for the particular genes being studied. Treatments

within the same sector had the same winning genotype, while treatments in different sectors had different winning genotypes.

As shown in Figure 1, the first principal component (PC1) and the second principal component (PC2) explained 97.9% of the variation, indicating that the biplot was sufficient to account for the differences in gene expression quantity and red light illumination treatment.

When considering the light treatment alone, the *Sgt3* markers were identified as the vertex genotype, falling into the fourth sector, which indicated significant gene expression under three different red light illumination durations (Figure 1c). As shown in Figure 1d, the varieties could be divided into two groups: D-6 and D-3 in the first sector; and JZ-12, L-6, and DXY as a separate group. The combination of the first principal component (PC1) and the second principal component (PC2) explained 96.1% of the variability. In D-6 and D-3, the *Pvs1* gene showed the highest expression level, while in JZ-12, L-6, and DXY, the expression of *Sgt3* was higher than that of other genes. The expression levels of *Hmg1*, *Hmg2*, *Pss1*, and *Sgt2* in the five genotypes were significantly lower than the expression levels of *Pvs1*, *Sgt1*, and *Sgt3*.

3.3. The Ranking of Seven Genes for Transcriptional Expression

The red line passing through the biplot origin is called the average treatment axis (ATA) and represents the mean level of expression. The blue lines that are close to the concentric circle indicate a higher mean expression level. The blue double-arrowed line represents the average treatment coordination (ATC) ordinate, which indicates greater variability (smaller stability) in either direction (Figure 2). Therefore, if the vector of a gene on the ATC abscissa is shorter, it indicates higher gene stability and less influence of the treatment on gene expression performance.

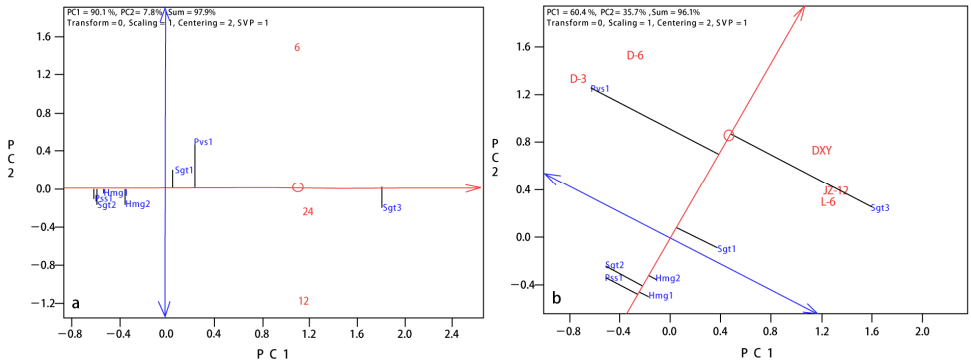

Figure 2. The GGE Biplot analysis showing the ranking of seven genes for the transcriptional expression across red light induction durations and genotypes. (**a**) Ranking of average gene expression across light illumination durations. (**b**) Ranking of average gene expression across genotypes. The numbers 6, 12, and 24 represent the three illumination durations; D-3, D-6, DXY, JZ-12 and HA represent genotypes involved in the experiment. *Pvs1*, *Sgt1*, *Sgt2*, *Sgt3*, *Pss1*, *Hmg1*, and *Hmg2* represent seven target enzymes involved in the SGA biosynthetic pathway. The plot is based on gene centering (center = 2) with SD scaling and no transformation of the data (scaling = 1, transformation = 0) and is genotype metric-preserving (SVP = 1).

In the biplot graph, stable genes should be near the average treatment (center of the small circle in Figure 2) and have the shortest vector from the ATC abscissa. The expression of *Sgt3* across the three red light illumination durations was higher than the average value. The vector of the *Sgt3* gene on the ATC abscissa was shorter, indicating higher gene stability and less influence of the three illumination treatments on gene expression performance (Figure 2a).

When considering only genotype differences, the expression level of *Sgt3* was still higher than that of other genes. The vector of the *Sgt3* gene on the ATC abscissa was longer, indicating lower expression stability of the *Sgt3* gene across the five genotypes (Figure 2b).

3.4. The Relationships among Red Light Illumination Durations and Genotypes

The concept of the "ideal test treatment" refers to a treatment that is both highly discriminatory and representative of all treatments [16]. In Figure 2a, the ideal test treatment is indicated by the center of the concentric circles on the ATC (Additive Main Effects and Multiplicative Interaction—Biplot). The distance between the ideal test treatment and the origin of the biplot represents the longest vector among all treatments. In this study, the point corresponding to 24 h of red light illumination was the closest to the center of the concentric circles, suggesting that it can be considered one of the best red light illumination durations for future gene expression analysis. On the other hand, the points corresponding to 6 and 12 h of red light illumination were relatively similar and not as effective for the seven genes analyzed (Figure 3a).

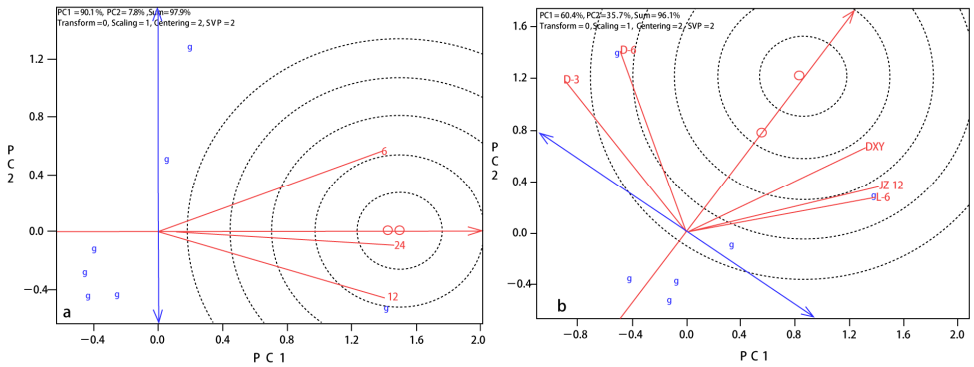

Figure 3. The GGE Biplot analysis showing the relationships between red light induction durations and genotypes. (**a**) Relationship between light illumination durations; (**b**) relationship among genotypes. The numbers 6, 12, and 24 represent the three illumination durations; D-3, D-6, DXY, JZ-12 and HA represent the genotypes involved in the experiment. *Pvs1*, *Sgt1*, *Sgt2*, *Sgt3*, *Pss1*, *Hmg1*, and *Hmg2* represent seven target enzymes involved in the SGA biosynthetic pathway. 'g' is used to show the distribution of genotypes on the GGE Biplot graph. The plot is based on gene centering (center = 2) with SD scaling and without transformation of data (scaling = 1, transform = 0), and it is gene metric-preserving (SVP = 2).

Regarding the test genotypes, genotype DXY was the closest to the center of the concentric circles on the ATC, indicating that it can be considered as one of the best genotypes for future gene expression analysis (Figure 3b).

3.5. The Relationships among Genotypes and Illumination Durations

The angle between the L-6, JZ-12, and 12 h vectors was less than 90°, indicating a high correlation between the expression of seven genes in the L-6 and JZ-12 tubers after 12 h and 6 h of red light illumination, respectively. Similarly, the genotype DXY and 24 h of red light illumination showed a high correlation as the angle between them was less than 90° (Figure 4a).

The markers of genotype L-6 represent the vertex genotype and fall into the third sector, indicating that the seven genes can be significantly expressed under 12 h and 6 h of red light illumination. Similarly, the markers of genotype DXY fall into the second sector, indicating that the seven genes can be significantly expressed under 24 h of red light illumination (Figure 4b).

Vertex genotype D-3 was not the winning genotype in any of the red light illumination treatments. Under the three red light illumination durations, the gene expression under L-6 was greater than the average value. The accumulation of SGAs (steroidal glycoalkaloids) did not occur under the three red light illumination durations for genotype D-3 (Figure 4c).

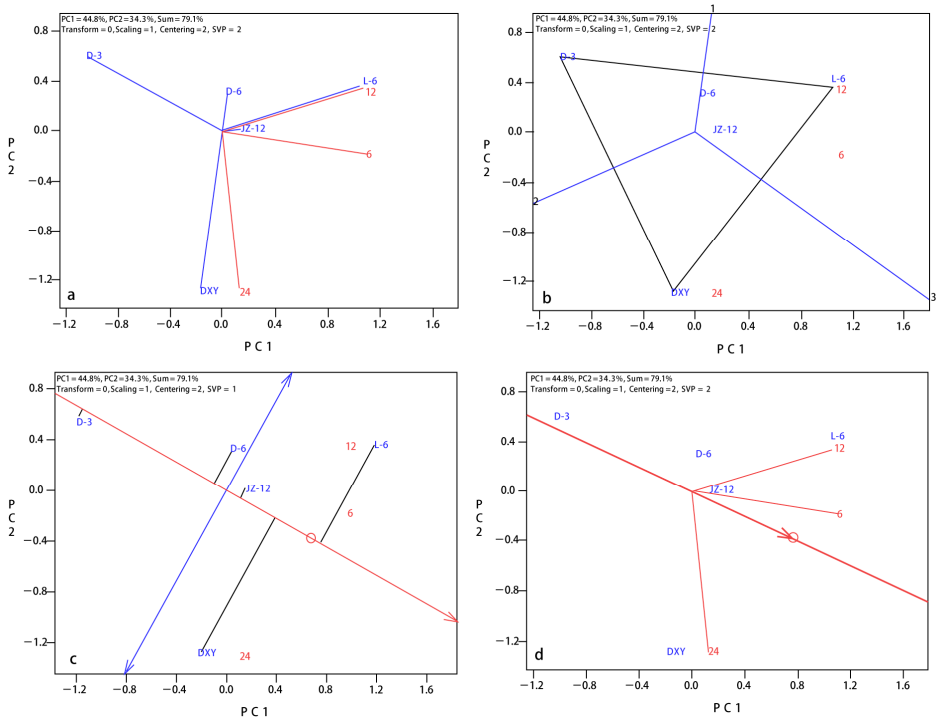

Figure 4. The GGE Biplot analysis showing genotype vs. illumination duration based on the mean value of red light-induced expression of seven genes. (**b**) The polygon view of the interaction between genotypes and red light illumination duration. (**d**) Representative comparison of different red light exposure times on gene expression. The numbers 6, 12, and 24 represent the three illumination durations; D-3, D-6, DXY, JZ-12 and L-6 represent genotypes involved in the experiment. *Pvs1*, *Sgt1*, *Sgt2*, *Sgt3*, *Pss1*, *Hmg1*, and *Hmg2* represent seven target enzymes involved in the SGA biosynthetic pathway. In (**a**,**b**,**d**), the plot is based on gene centering (center = 2) with SD scaling and without transformation of data (scaling = 1, transform = 0), and it is gene metric-preserving (SVP = 2). In Figure 4c, the plot is based on gene centering (center = 2) with SD scaling and without transformation of data (scaling = 1, transformation = 0), and it is genotype metric-preserving (SVP = 1).

As shown in Figure 4a, the line connecting the time and origin is called the treatment vector. The length of the vector reflects the discrimination of different treatments on gene expression ability. The length of each treatment vector is closed, indicating that the expression of each gene for different treatment times has similar trends. The angle between the vector and the average treatment axis (ATA) reflects the treatment representativeness, with a smaller angle indicating stronger representativeness. In Figure 4d, it should be noted that red light illumination for at least 6 h results in a higher level of gene expression for all genotypes.

The angles of the vectors were acute, indicating that red light-induced gene expression has the same pattern at each time point. The angle between the vector of 6 h and the ATC abscissa was the smallest among the three treatment durations, indicating that the pattern of induced gene expression after 6 h of treatment is more representative than those of 12 and 24 h.

4. Discussion

It should be noted that this study only investigated the differential expression profiles of the seven key genes involved in SGA biosynthesis in potato tubers under red light illumination for five potato cultivars. However, it is important to acknowledge that we did not investigate the differential expression profiles of the seven key genes involved in SGA biosynthesis in potato stems, leaves, and other parts of potato under red light illumination

for the five potato cultivars. Additionally, other light treatments could also be selected as experimental factors.

Previous studies have suggested that levels of SGAs in tubers and leaves may respond to certain stresses and environmental conditions. For example, Hamouz et al. [26] found that the glycoalkaloid content in potato tubers varied with cultivar, flesh color, location, and year of cultivation. Zarzecka et al. [27] found that the level of glycoalkaloids in leaves and tubers was influenced by the use of herbicides, which significantly increased or decreased the SGA content compared to control tubers. Changes in SGA content were detectable after wounding and exposure to light. Nahar et al. [28] indicated that increased levels of SGAs in potato tubers during wounding and light exposure were mediated by the coordinated expression of key genes involved in isoprenoid and steroid metabolisms. These findings suggest that differences in gene expression levels may contribute to variations in SGA levels among cultivars.

In our research, the obtuse angles between the expression of *Hmg1*, *Pss1*, *Sgt2*, *Hmg2*, and red light illumination treatments with different genotypes indicated a negative correlation between them. The expression levels of *Hmg1*, *Hmg2*, *Pss1*, and *Sgt2* in the five genotypes were significantly lower than the expression levels of *Pvs1*, *Sgt1*, and *Sgt3*. This is consistent with the findings of Manjulatha et al. [29], who reported that the expression levels of *Hmg1* and *Pss1* can be used as selection markers for breeding potato cultivars with low levels of SGAs.

Light quality and intensity have a severe impact on SGA biosynthesis, causing quality degradation in potato by greening and accelerating SGA-induced toxicity. In our research, we used red light with three illumination durations and five potato cultivars. We concluded that seven genes can be significantly expressed in the L-6 genotype under 12 h and 6 h of red light illumination. Other studies have shown that yellow light can be used as an alternative light source and showed lower transcript levels of key genes for SGA accumulation compared to fluorescent light. These studies used the Atlantic and Haryoung cultivars as experimental materials and observed different responses to seven light qualities [2,30].

GGE biplots are a useful tool for analyzing MET data and visualizing other types of two-way data. In this paper, we innovatively used GGE biplots to analyze the expression of seven genes regulating the glucoside alkaloid synthesis pathway in the tubers of five potato varieties under different red light illumination durations. This analysis involved a total of seven genes and three illumination durations, making the analysis of gene expression levels complex. GGE biplots can solve these complex problems and provide convenience for data analysis. Biplots not only generate a large number of icons but also help identify the relationship between gene expression levels, genotypes, and illumination durations.

As shown in Figure 2, the expression levels of *Pvs1*, *Sgt1*, and *Sgt3* were much higher than those of the other four genes in all genotypes. This suggests that the expression levels of these three genes are generally higher than those of other genes involved in the regulation of glycosidic alkaloid synthesis. However, it is important to determine the content of different glucoside alkaloids in the tubers of the five genotypes to confirm this trend. From the perspective of expression stability under the three illumination durations, the expression of the *Sgt3* gene is not only high but also relatively stable compared to that of *Pvs1* and *Sgt1*. However, an opposite result was observed for the *Sgt3* gene across the five genotypes. The main function of *Sgt3* is to catalyze the synthesis of the corresponding form by β-chaffinine and β-solanine. The expression of *Sgt3* shows little variation across different illumination durations, but the duration of red light treatment may have a greater influence on its expression level. From the perspective of genotypes, the expression levels of all genes in L-6 and DXY were higher and consistent in their expression trend. This is consistent with the characteristics of these two genotypes. L-6 is a wild type with a higher glycosidic alkaloid content in tubers than common cultivated varieties, while the cultivar DXY has good disease resistance and taste due to its higher glycosidic alkaloid content compared to other local cultivations.

5. Conclusions

The expression levels of *Pvs1*, *Sgt1*, and *Sgt3* were found to be significantly higher than those of the other tested genes. Among these genes, *Sgt3* showed high and stable transcriptional expression under three different durations of red light illumination. However, it showed less stability across different genotypes. Similar expression patterns were observed for all seven genes in both the 12 and 24 h treatments. Notably, it is important to note that a minimum of 6 h of red light illumination was required for the optimal gene expression in the DXY genotype. In the L-6 genotype, significant expression of all seven genes was observed after 12 and 6 h of red light exposure.

Therefore, the results of this study demonstrate the utility of GGE biplots as a valuable tool for analyzing and visualizing the differential expression profile of these seven key genes in potato tubers under red light illumination. Additionally, the quantitative data presented in this study elucidate the relationship between different treatments and genotypes. The results of this research provide important insights for future studies on the biosynthesis of SGAs and highlight the potential wide application of GGE biplots in various research fields beyond crop breeding.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13082127/s1, Figure S1:Simplified biosynthetic pathway for steroidal glycoalkaloids; Table S1: Primer information for qRT—PCR/.

Author Contributions: Conceptualization, Y.L., T.C., and J.B.; methodology, F.Z.; software, F.Z. and J.B.; validation, T.C. and J.B.; formal analysis, F.Z.; investigation, Y.L., T.C., and J.B.; resources, J.B.; data curation, Y.L. and T.C.; writing—original draft preparation, Y.L.; writing—review and editing, F.Z.; visualization, F.Z.; supervision, Y.L.; project administration, Y.L. and J.B.; funding acquisition, Y.L. and J.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Program for Changjiang Scholars and Innovative Research Team in University, grant number IRT13019; the Technology Innovation Guidance Program of Gansu Province-Agriculture, grant number 22CX8NJ153, the National Natural Science Foundation of China, grants number 31660432, 31460369, the International Science and Technology Cooperation Program of China, grant number 2014DFG31570, and the National Modern Agriculture Industry Technology System Construction Special Funds, grant number CARS-10.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the data from this trial are being used in ongoing more in-depth studies.

Acknowledgments: We thank all staff working in these experimental sites for their helpful assistance. We also thank Jiangping Bai for critical comments and editorial assistance in improving the language of the article.

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

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