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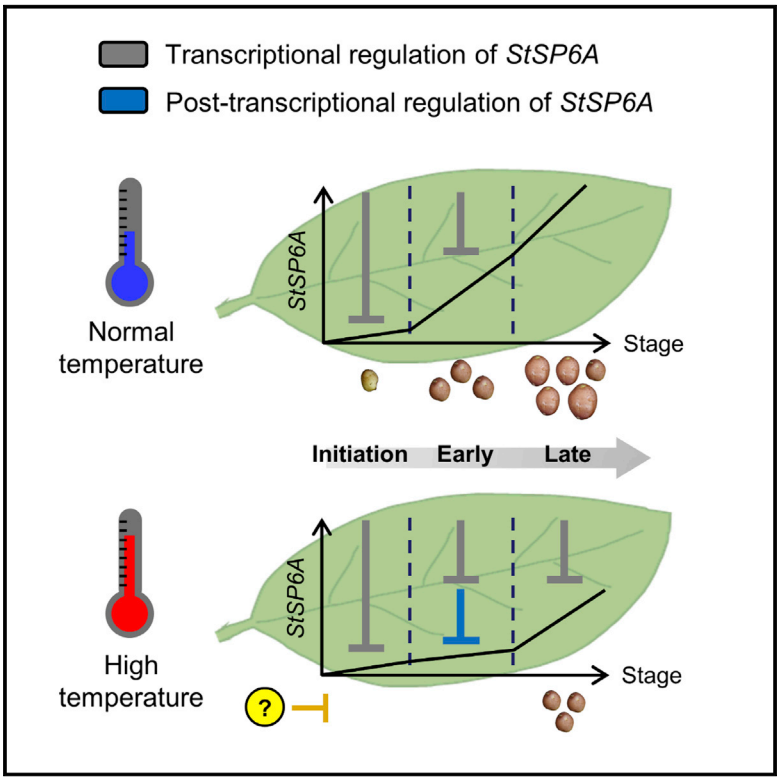
Temporally distinct regulatory pathways coordinate thermo-responsive storage organ formation in potato

Graphical abstract



Highlights

- *StSP6A* is post-transcriptionally regulated by heat at the early tuberization stage
- *StSP6A* is transcriptionally suppressed by heat at the late tuberization stage
- Upregulation of *StSP6A* restores suppressed tuber formation by heat
- Additional regulators are involved in the heat suppression of tuber yield

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In brief

Park et al. show that transcript levels of FT homolog *StSP6A* are suppressed by different regulatory pathways at the early and late stages to inhibit tuber formation under high-temperature conditions in potato. This study reveals that potato has temporally distinct molecular mechanisms that finely control tuberization at high temperatures.

Park et al., 2022, Cell Reports *38*, 110579
March 29, 2022
https://doi.org/10.1016/j.celrep.2022.110579

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Temporally distinct regulatory pathways coordinate thermo-responsive storage organ formation in potato

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https://doi.org/10.1016/j.celrep.2022.110579

SUMMARY

Tuberization is an important developmental process in potatoes, but it is highly affected by environmental conditions. Temperature is a major environmental factor affecting tuberization, with high temperatures suppressing tuber development. However, the temporal aspects of thermo-responsive tuberization remain elusive. In this study, we show that FT homolog StSP6A is suppressed by temporally distinct regulatory pathways. Experiments using *StSP6A*-overexpressing plants show that post-transcriptional regulation plays a major role at the early stage, while transcriptional regulation is an important late-stage factor, suppressing *StSP6A* at high temperatures in leaves. Overexpression of *StSP6A* in leaves restores tuber formation but does not recover tuber yield at the late stage, possibly because of suppressed sugar transport at high temperatures. Transcriptome analyses lead to the identification of potential regulators that may be involved in thermo-responsive tuberization at different stages. Our work shows that potato has temporally distinct molecular mechanisms that finely control tuber development at high temperatures.

INTRODUCTION

Potato (*Solanum tuberosum* L.) develops a specialized storage organ from underground stems called a tuber. Tuber formation is affected by various environmental factors, including temperature, photoperiod, and salinity (Gururani et al., 2013; Hancock et al., 2014; Lehretz et al., 2019; Martinez-Garcia et al., 2002; Morris et al., 2019; Pumisutapon and Topoonyanont, 2017). High temperature is a major negative environmental factor in tuberization. Elevated temperature during the potato-growing season causes severe crop loss (Hijmans, 2003). Previous studies have used various potato cultivars to examine the effects of high temperature on tuber development and to understand the mechanisms behind tuberization temperature responses. High temperature leads to decreased tuber yield in *Solanum tuberosum* cvs. Desiree, Spunta, Solara, and Kufri Chandramukhi (Van Dam et al., 1996; Lehretz et al., 2019; Singh et al., 2015), suggesting that the effects of temperature on tuberization are conserved in potato cultivars. Studies have shown that expression of genes related to tuberization is altered by temperature changes; elevated growth temperature upregulates the expression of genes encoding SELF-PRUNING 5G (StSP5G), CONSTANS-LIKE 1 (StCOL1), and GIGANTEA (Hancock et al., 2014; Singh et al., 2015), which are involved in the inhibition of tuberization (Abelenda et al., 2016; Gonzalez-Schain et al., 2012). On the other hand, high temperature negatively regulates the expression of a gene encoding SELF-PRUNING 6A (StSP6A), which plays an important role in inducing tuber formation (Hancock et al., 2014; Navarro et al., 2011; Singh et al., 2015). These findings suggest that potato has developed diverse regulatory pathways that finely control tuberization in response to temperature fluctuations.

Potato tuberization is mainly controlled by StSP6A, which is a homolog of FLOWERING LOCUS T (FT), an *Arabidopsis* florigen (Navarro et al., 2011). StSP6A is identified as responsible for photoperiod-dependent tuber development. In *Solanum tuberosum* group Andigena, which specifically develops tubers during short days (SD), *StSP6A* expression during SD is increased in both leaves and stolons (Navarro et al., 2011). Overexpression of *StSP6A* induces rapid tuberization and increased tuber yield, but suppression of *StSP6A* expression causes reduced tuberization, indicating the critical role of StSP6A in tuber development (Lehretz et al., 2019; Navarro et al., 2011). Photoperiod-dependent expression of *StSP6A* is controlled by another FT-clade member, StSP5G, which is a negative regulator of StSP6A (Abelenda et al., 2016). Molecular genetic studies have additionally

identified that StCOL1, a homolog of *Arabidopsis* CONSTANS, is an upstream transcriptional regulator of StSP5G (Abelenda et al., 2016). Notably, the StCOL1-StSP5G regulatory module functions in leaves; thus, it is suggested that transcriptional regulation of *StSP6A* primarily occurs in leaves and then StSP6A is transported through the phloem as a mobile signal to induce tuberization (Abelenda et al., 2016; Navarro et al., 2011). Another molecular factor affecting tuber development is gibberellins (GAs). A decrease in bioactive GA levels by overexpression of the *GA2 oxidase 1* (*StGA2ox1*) gene in potato induces tuber formation and swelling (Dong et al., 2017; Shi et al., 2016), suggesting that GAs are negative regulators of tuberization. It is predicted that StSP6A may regulate Gas, because overexpression of *StSP6A* induces *StGA2ox1* expression (Kloosterman et al., 2007; Navarro et al., 2011). However, the detailed relationship between StSP6A and GAs remains undetermined.

Recent studies have reported that StSP6A is also involved in the temperature dependency of tuber development. In *Solanum tuberosum* cv. Desiree, *StSP6A* expression is decreased in plants grown at high temperatures in comparison to that in plants grown under normal conditions (Hancock et al., 2014; Lehretz et al., 2019; Morris et al., 2019). Upstream regulators controlling *StSP6A* expression at high temperatures have been suggested. The potato homolog of the *Arabidopsis* clock gene TIMING OF CAB EXPRESSION 1 (TOC1), StTOC1, is a thermo-responsive transcriptional regulator that suppresses *StSP6A* expression (Hancock et al., 2014; Morris et al., 2019). Silencing *StTOC1* expression increases both tuber yield and *StSP6A* transcript levels. The presence of TOC1 recognition motifs in the promoter regions of the *StSP6A* locus implies that StTOC1 could directly bind to *StSP6A* promoters (Morris et al., 2019). Meanwhile, post-transcriptional regulation of *StSP6A* transcripts is identified by investigating a codon-optimized version of *StSP6A* in *Solanum tuberosum* cv. Solara (Lehretz et al., 2019). A microRNA (miRNA) named SUPPRESSING EXPRESSION OF SP6A (SES) is predicted to target *StSP6A* transcripts. Overexpression of *SES* reduces *StSP6A* transcript levels but does not affect the codon-optimized version of *StSP6A*, indicating that SES is a negative regulator of *StSP6A*. Notably, the expression of *SES* is increased by heat treatment, and sequestering the small RNAs enhances tuber yield at high temperatures. This suggests that post-transcriptional regulation of *StSP6A* is important for tuber development under high-temperature conditions.

In this study, we adopted a time course analysis of potato tuberization pathways at high temperatures. At the early stage of tuberization, constitutive overexpression of *StSP6A* did not fully overcome the effects of high temperature on tuber development. Consistently, *StSP6A* transcript levels were downregulated by high-temperature treatment even under the control of a constitutive promoter, suggesting that *StSP6A* is regulated post-transcriptionally at the early stage under high-temperature conditions. Constitutive overexpression of *StSP6A* restored thermo-inhibition of *StSP6A* transcript levels in leaves at the late stage, indicating that transcriptional regulation plays a major role at the late stage. In addition, transcriptome analysis showed that genes involved in tuber development exhibited different expression patterns between the early and late stages after high-temperature treatment. Our work showed that potato has temporally distinct regulatory pathways, possibly for fine and multi-level control of tuber development at high temperatures.

RESULTS

Suppression of potato tuberization at high temperature

Many potato tuberization studies have been performed, but the effects of periods of high temperature on tuber development are poorly understood. We therefore treated potato plants with high temperatures for different time periods after transfer from Murashige and Skoog (MS)-agar medium to soil. Plants were grown under neutral days (12 h light:12 h dark) at either normal temperatures (NT; 22°C light:16°C dark), slightly high temperatures (SHT; 30°C light:24°C dark), or high temperatures (HT; 35°C light:29°C dark) for 5 and 9 weeks. We did not grow potatoes for more than 9 weeks because many leaves showed senescence at HT (Figure 1A). At 5 weeks, whereas tubers were formed at NT and SHT, they were not observed at HT (Figures 1B and 1C). Tuber numbers from plants grown at SHT were significantly lower than those grown at NT (Figure 1C). Measurement of stolon yield and number showed that HT treatment significantly reduced both yield and number, while SHT treatment showed intermediate effects (Figure 1D). At 9 weeks, tuber yield decreased with increasing temperature, but tuber numbers were similar at SHT and HT (Figure 1C). Stolon yield and number were decreased by both SHT and HT treatment, and there was no difference between the values at SHT and HT (Figure 1D).

Because tuber weight and size were diverse in each treatment, we analyzed variations by measuring the percentage of the number of tubers that belonged to each weight or width range. Visualization of the results using a heatmap showed that tuber weights and sizes at 9 weeks were more diverse in plants grown at NT than those at SHT and HT (Figures 1E and 1F). Whereas plants grown at NT developed both small and large tubers, the percentage of small tubers within the range of 0–2 g or 0.4–1.3 cm at SHT and HT was relatively lower than that at NT. These results suggest that high temperature suppresses tuber development, particularly the formation of new tubers during the tuberization process.

The absence of tubers in plants grown at HT for 5 weeks led us to hypothesize that high temperature delays initiation of tuberization. We thus examined tuber and stolon formation at earlier time points. Plantlets transferred from the MS-agar medium to soil started tuberization at 3 weeks under NT, but those grown at SHT initiated tuber formation at 4 weeks (Figures 2A and 2B). Plants grown at HT did not start tuberization until 4 weeks. Similarly, measurement of the stolon numbers showed that higher temperatures caused a more severe delay to stolon formation (Figures 2A and 2C). These data indicate that initiation of both tuber and stolon formation is delayed by high temperatures.

Effects of *StSP6A* overexpression on early- and late-stage tuberization at high temperature

Previous studies have shown that StSP6A plays a key role in regulating potato tuberization (Lehretz et al., 2019; Navarro et al., 2011). In addition, it has been shown that expression of *StSP6A* is suppressed by high temperatures (Hancock et al., 2014; Lehretz et al., 2019; Singh et al., 2015). These reports

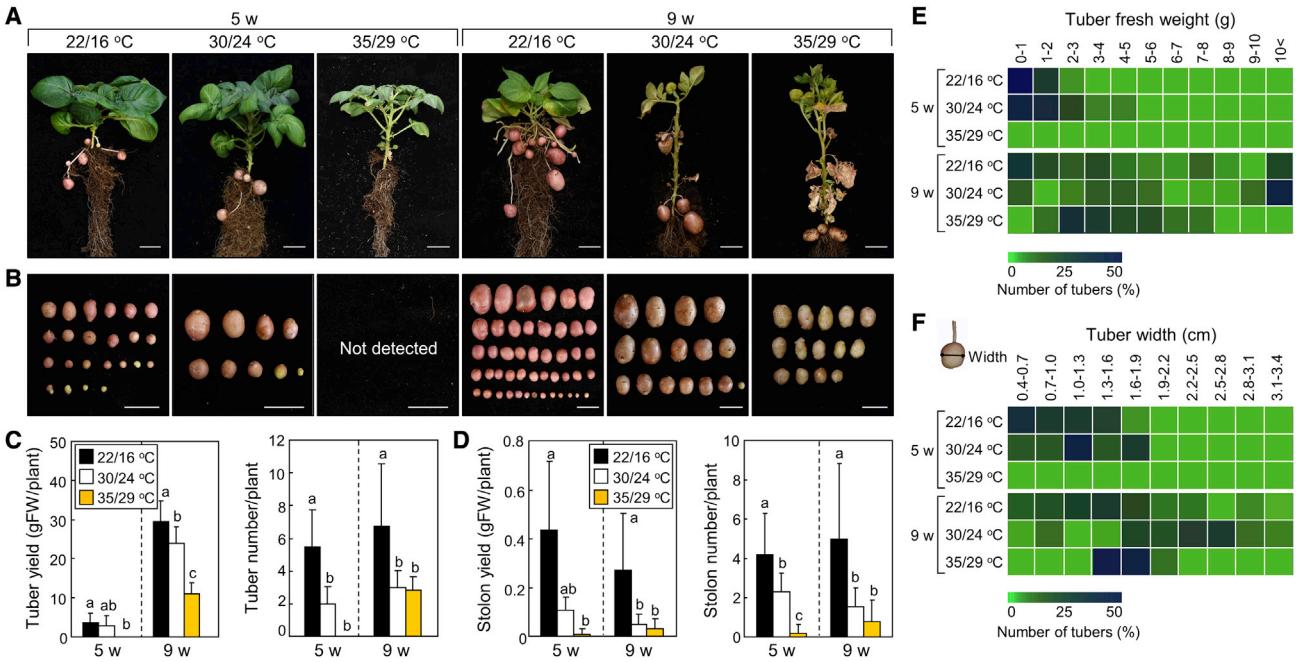

Figure 1. Potato tuberization is suppressed at high temperature

For preparing potato plantlets, wild-type plantlets containing 2–3 nodes were grown under *in vitro* conditions for 1 week in MS-agar plates. Plantlets were transferred to soils and grown at different temperatures for 5 and 9 weeks under neutral days (12:12-h light-dark) in pots. Letters indicate groups that are statistically significantly different from each other ($p < 0.05$, Tukey's test); w, weeks.

(A and B) Phenotypes of potato plants. Photographs of whole plants (A) and total tubers harvested from 5 plants (B) were taken at 5 and 9 weeks after the plantlets were transplanted. Scale bars, 3 cm.

(C) Measurement of tuber productivity at different temperatures. Tuber yield (left) and tuber number (right) per plant were measured at 5 and 9 weeks. Five replicates were averaged and statistically analyzed. Error bars indicate ±SD.

(D) Measurement of stolon yield (left) and stolon number (right) per plant. Five replicates were averaged and statistically analyzed. Error bars indicate ±SD.

(E and F) Percentage of tuber numbers among total number of tubers in the annotated range of fresh weight (E) or width (F) was displayed using heatmap. Total number of tubers is the sum of tuber numbers in all analyzed potato plants at each temperature and time point.

suggest that the thermal sensitivity of *StSP6A* transcripts is a part of the mechanism behind suppression of tuberization at high temperatures. Therefore, we analyzed time course expression of *StSP6A* at different temperatures. Expression of *StSP6A* gradually increased at NT, but that was significantly reduced by exposure to HT in both leaves and stolons from 4 to 8 weeks (Figures 3A and 3B). Together with the known role of StSP6A in tuberization, these results imply that reduced expression of *StSP6A* at SHT and HT affects suppressed tuber development at high temperatures. However, expression of *StSP6A* cannot fully explain thermo-regulation of tuberization, because exposure to high temperature delayed initiation of tuberization at 3 weeks when expression of *StSP6A* was not altered (Figures 2 and 3A).

To examine the relationship between *StSP6A* expression and tuberization at different temperatures, we generated transgenic lines expressing the *StSP6A* gene under the control of the CaMV 35S promoter (Figure 3C). Three independent *35S*:*StSP6A* transgenic lines were grown at NT and HT because the effects of SHT on tuberization were less obvious than those of HT (Figure 1). Both wild-type and transgenic plants exhibited increased plant height at HT (Figure S1), which is related to the thermo-morphogenesis phenotype (Quint et al., 2016). Overexpression of *StSP6A* allowed the formation of tubers at HT after 5 weeks, but there were still significant differences between NT and HT in both tuber number and yield (Figures 3D, 3E, and S2A). Stolon number and yield were also significantly decreased in both wild-type and *35S*:*StSP6A* transgenic plants, indicating that overexpression of *StSP6A* using 35S promoter cannot fully restore stolon and tuber development at 5 weeks under HT conditions. Initiation of tuberization in *35S*:*StSP6A* plants was analyzed at 3 weeks under NT and HT conditions, and the results showed that tuberization was largely delayed in *35S*:*StSP6A* at HT (Figure S3).

We next analyzed tuberization of *35S*:*StSP6A* at 9 weeks under NT and HT conditions. Notably, *35S*:*StSP6A* plants exhibited similar tuber number at NT and HT, whereas the tuber numbers of wild types were significantly reduced at HT (Figures 3F and 3G). In addition, stolon number and yield were restored by overexpressing *StSP6A* at HT. However, tuber yield was still significantly decreased at HT in both wild-type and *35S*:*StSP6A* transgenic plants (Figure 3G). Consistent with the tuber yield data, the tuber/shoot ratio was also decreased at HT in both the wild-type and the transgenic plants (Figure S4). These results suggest that constitutive overexpression of *StSP6A* using the 35S promoter completely restored tuber formation, but it could not improve tuber yield at the late stage under HT conditions.

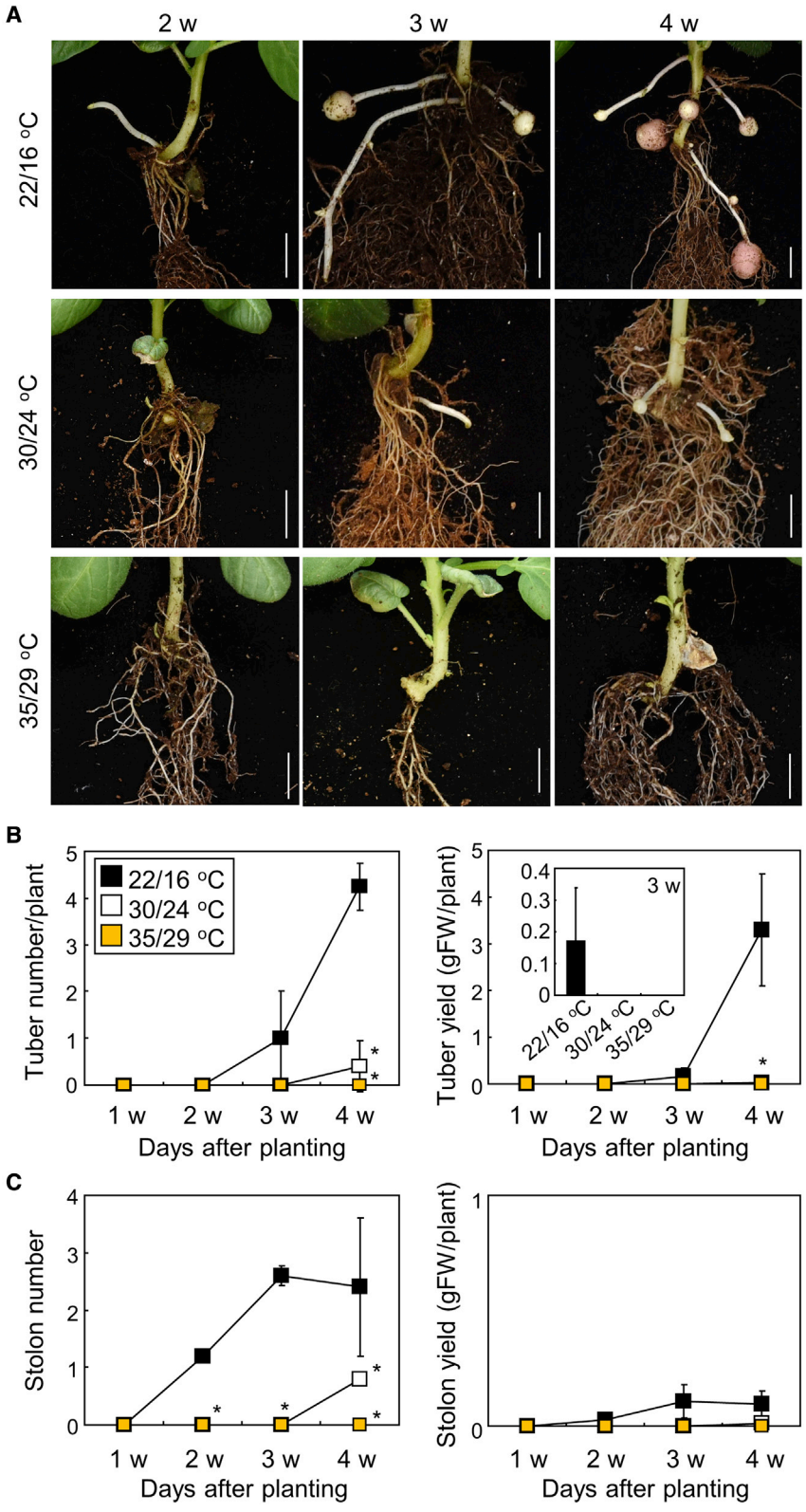

Figure 2. Initiation of tuberization is delayed at high temperature

Preparation of potato plantlets was performed as described in Figure 1. Plantlets were transferred to soil and grown at different temperatures for up to 4 weeks under neutral days conditions.

(A) Phenotypes of underground part in potato plants. Potato plants were grown for either 2, 3, or 4 weeks at the indicated temperatures. Photographs were taken after removal of soils. Representative pictures among 5 replicates are displayed. Scale bars, 1 cm.

(B) Measurement of tuber productivity at different temperatures. Tuber number (left) and tuber yield (right) per plant were measured at the indicated time points after the plantlets were transferred to soil. Graph inset on the right shows enlarged view of tuber yield at 3 weeks. Five replicates were averaged and statically analyzed (t test, *$p < 0.05$ compared with 22/16°C). Error bars indicate ±SD.

(C) Measurement of stolon number (left) and stolon yield (right) per plant. Plants were grown as described in (B). Five replicates were averaged and statistically analyzed (t test, *$p < 0.05$). Error bars indicate ±SD.

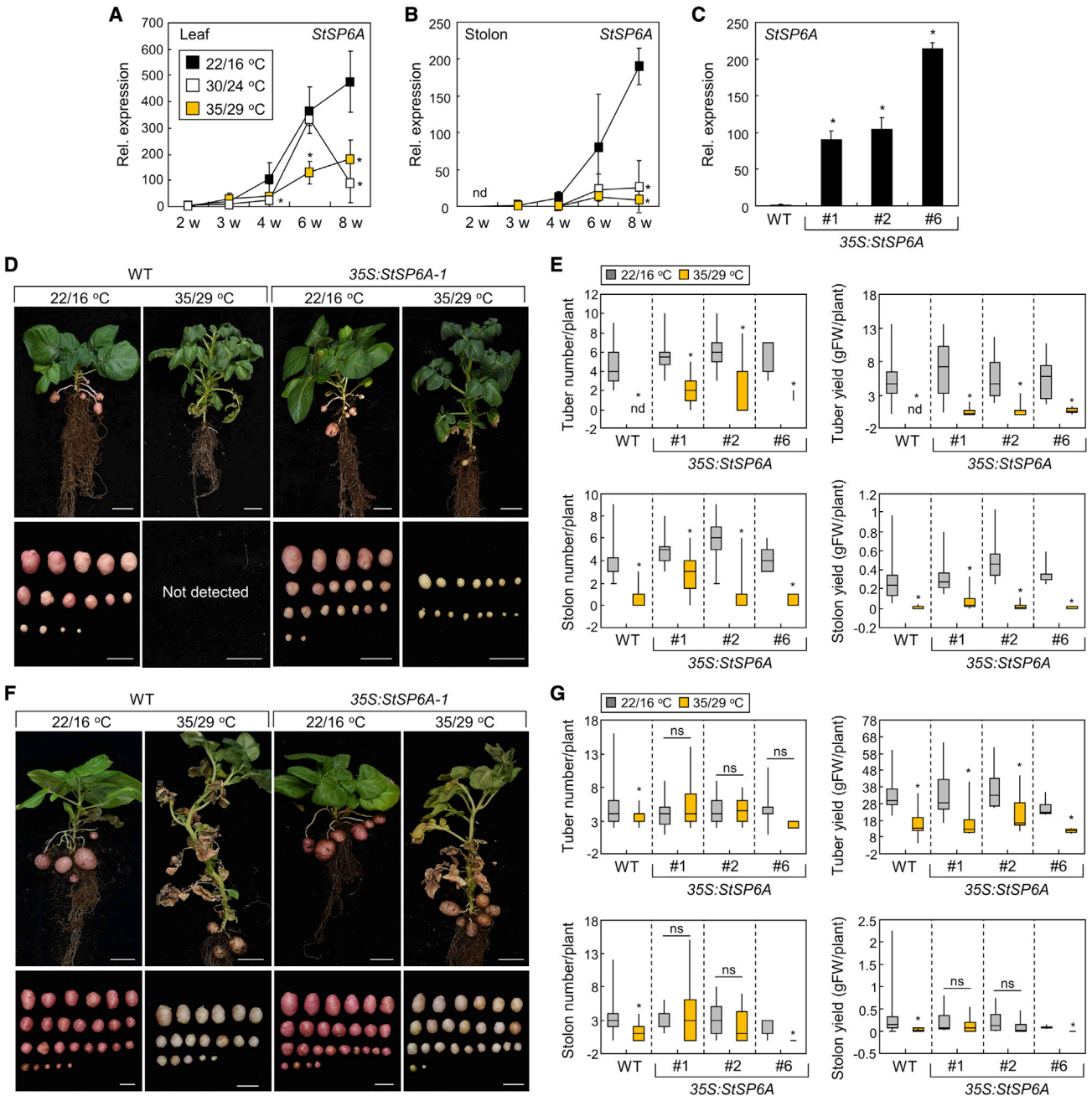

Figure 3. Tuberization of *35S:StSP6A* plants at different temperatures

Preparation of potato plantlets was performed as described in Figure 1. Plantlets were transferred to soil and grown at different temperatures for 5 or 9 weeks under neutral-days conditions. For (A), (B), (E), and (G), Student's t test was performed to determine statistical significance (*$p < 0.05$ compared with 22/16°C). Error bars indicate ± SD.

(A and B) Expression of *StSP6A* at different temperatures. Leaves (A) or stolons (B) were harvested at the indicated time points. Four to five biological replicates were analyzed; nd, not determined.

(C) Expression of *StSP6A* in *35S:StSP6A* transgenic plants. Wild-type (WT) and *35S:StSP6A* plantlets containing 2–3 nodes were grown under *in vitro* conditions for 1 week in MS-agar plates. Leaves were harvested to analyze gene expression. Three technical replicates were averaged (t test, *$p < 0.05$ compared with WT).

(D–G) Phenotype of *35S:StSP6A* transgenic plants at 5 and 9 weeks at different temperatures. Plants were grown for 5 (D and E) or 9 weeks (F and G) at different temperatures. Photographs were taken after removal of soils (D and F). Tuber and stolon productivities were measured (E and G). For WT, experiments were performed 4 times; for *35S:StSP6A-1*, and *-2*, experiments were performed 3 times; and for *35S:StSP6A-6*, a single experiment was performed. Four to five replicates were used for each experiment. All the experimental data were averaged and statistically analyzed. Scale bars, 3 cm; ns, not significant.

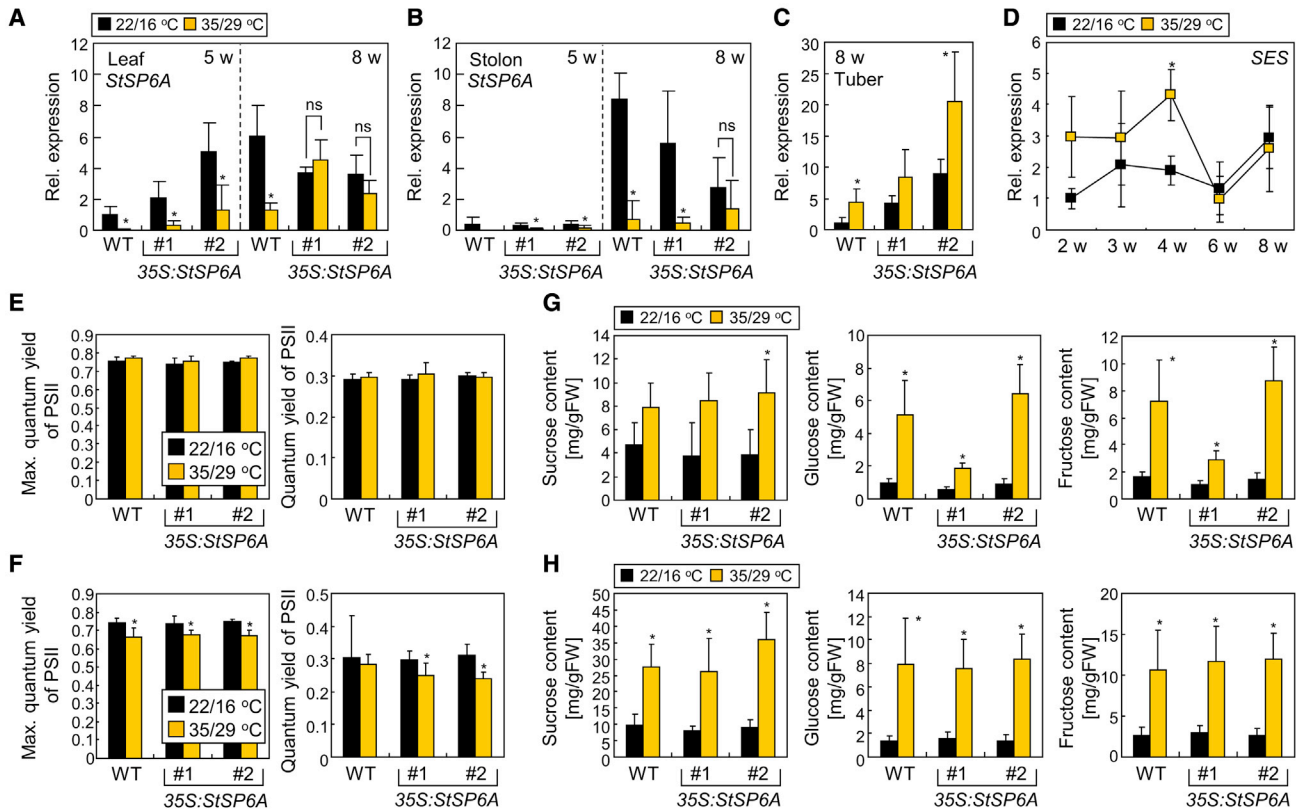

Figure 4. Analysis of *StSP6A* expression and sugar contents in *35S:StSP6A* plants at different temperatures

WT and *35S:StSP6A* plants were grown as described in Figure 1. Error bars indicate ±SD.

(A–C) Expression of *StSP6A* at different temperatures. Leaves (A), stolons (B), and tubers (C) of plants grown at different temperatures were harvested at the indicated time points. Three to 15 replicates were averaged and statistically analyzed (t test, *p < 0.05 versus 22/16°C).

(D) Abundance of SES miRNA during tuberization at different temperatures. WT plants were grown for the indicated time periods at different temperatures. Leaves were harvested, and the abundance of SES was analyzed. Three to five replicates were averaged and statistically analyzed (t test, *p < 0.05 compared with 22/16°C).

(E and F) Measurement of photosynthetic performance using chlorophyll fluorescence. Plants were grown at different temperatures for 5 (E) and 9 weeks (F). Chlorophyll fluorescence was measured using leaves. Four to eight replicates were averaged and statistically analyzed (t test, *p < 0.05 compared with 22/16°C).

(G and H) Measurement of sugar contents. Plants were grown at different temperatures for 5 (G) and 9 weeks (H). Sucrose, glucose, and fructose levels in the leaves were measured using HPLC. Three to eight replicates were averaged and statistically analyzed (t test, *p < 0.05 compared with 22/16°C).

Post-transcriptional and transcriptional regulation of *StSP6A* during tuberization at high temperatures

To investigate the different effects of *StSP6A* overexpression at different time points, we analyzed expression of *StSP6A* in *35S:StSP6A* transgenic plants at 5 and 8 weeks. Because regulation of *StSP6A* expression in leaves is closely related to tuber formation (Abelenda et al., 2016; Lehretz et al., 2019), we first analyzed the expression in leaves. Although *StSP6A* was overexpressed using the 35S promoter, expression of *StSP6A* was significantly decreased by HT at 5 weeks (Figure 4A). By contrast, reduced *StSP6A* expression at HT was fully restored by *StSP6A* overexpression at 8 weeks. These results are consistent with the data on the effects of HT on tuber number (Figures 3E and 3G), showing that *StSP6A* expression in leaves affects tuber formation at HT. Contrary to the results of the leaves, expression of *StSP6A* at HT was not restored in the *35S:StSP6A* stolons at 8 weeks (Figure 4B). In the tubers, *StSP6A* expression was increased at HT in the wild-type plants, and similar expression patterns were observed in the *35S:StSP6A* transgenic plants (Figure 4C).

Different expression patterns of *StSP6A* in *35S:StSP6A* leaves at different time points under HT conditions led us to hypothesize that *StSP6A* transcript levels are post-transcriptionally regulated at the early stage (~5 weeks) but are transcriptionally controlled at the late stage (~8 weeks) under HT conditions. A recent study identified that the miRNA SES acts as an upstream regulator of *StSP6A* by cleavage of *StSP6A* transcripts at high temperatures (Lehretz et al., 2019). To examine the relationship between SES and *StSP6A* transcripts, the abundance of SES during tuberization at different temperatures was analyzed in leaves. Consistent with the *StSP6A* transcript levels, SES abundance was increased at HT until 4 weeks and then reduced to a level comparable to that at NT after 6 weeks (Figure 4D). We analyzed SES abundance in *35S:StSP6A* plants at 8 weeks under NT conditions because *StSP6A* expression was not increased in the transgenic plants at NT (Figure 4A), but SES abundance was

not altered by *StSP6A* overexpression (Figure S5). Together, our data indicate that, in leaves, post-transcriptional regulation of *StSP6A* through small RNAs, including SES, plays a major role in thermo-suppression of *StSP6A* at the early stage of tuberization, whereas transcriptional regulation of *StSP6A* is critical at the late stage.

Analysis of photosynthetic parameters and sugar contents at different temperatures

In our data, overexpression of *StSP6A* restored decreased tuber number at the late stage of tuberization under HT conditions, but tuber yield was not recovered (Figure 3G). These results suggest that increased expression of *StSP6A* only improves tuber formation but not tuber growth. To understand the effects of high temperature on tuber yield, we analyzed photosynthetic parameters by measuring chlorophyll fluorescence. Maximum quantum yield of photosystem II (PSII) shows maximum quantum efficiency of open PSII, and quantum yield of PSII shows the proportion of the light energy used for photochemistry in PSII (Maxwell and Johnson, 2000). At 5 weeks, both maximum quantum yield of PSII and quantum yield of PSII were not altered by HT exposure (Figure 4E). At 9 weeks, maximum quantum yield of PSII was slightly reduced at HT in both wild-type and *35S*:*StSP6A* transgenic plants, whereas quantum yield of PSII was decreased only in the transgenic plants (Figure 4F). However, tuber yield was significantly reduced in wild-type and *35S*:*StSP6A* plants at both 5 and 9 weeks under HT conditions (Figures 3E and 3G), suggesting that photosynthetic parameters cannot explain the reduced tuber yield at HT.

Next, we analyzed the contents of sugars, which are final products of photosynthesis and are transported to tubers for starch biosynthesis (Abelenda et al., 2019). Notably, sucrose, glucose, and fructose contents in leaves were highly increased at HT (Figures 4G and 4H). The increase of sugar contents in leaves was more significant at 9 weeks than that at 5 weeks. No clear difference was observed in the sugar contents between wild-type and *35S*:*StSP6A* plants, suggesting that high-temperature-mediated accumulation of sugars in leaves is independent of StSP6A. Because defects in sugar transport result in accumulation of sugars in leaves with reduced tuber yield (Abelenda et al., 2019), it is possible that the decrease in tuber yield at HT is due to suppressed sugar transport from source to sink organs.

Temperature-responsive genes at different tuberization stages: Transcriptome analysis

Our data showed that, despite the delay in initiation of tuberization at 3 weeks caused by HT treatment, *StSP6A* transcript levels under NT, SHT, and HT conditions were similar at this time point (Figures 2 and 3A). In addition, overexpression of *StSP6A* partially restored tuber formation at 5 weeks under HT conditions (Figure 3E), but it could not affect thermo-suppression of tuber formation at 3 weeks (Figure S3). These results led us to hypothesize that there are genes other than *StSP6A* responsible for delayed tuberization at high temperatures during initiation. Because the expression of key genes involved in tuberization is regulated in leaves, with information then transported to the stolons (Abelenda et al., 2016; Navarro et al., 2011), we analyzed the transcriptome of potato leaves through RNA sequencing. We used leaves from plants grown at either NT or HT for 3 and 8 weeks. Principal component analysis (PCA) of the results clearly identified three groups: samples treated with NT for 3 weeks (3W NT), NT for 8 weeks (8W NT), and HT for 3 and 8 weeks (3W HT and 8W HT; Figure 5A). This analysis showed that the patterns of gene expression were largely different between 3 and 8 weeks under NT conditions but were similar between 3 and 8 weeks under HT conditions (Figure 5A).

Next, we found 1,447 and 5,571 thermo-responsive genes at 3 and 8 weeks, respectively, by analyzing differentially expressed genes (DEGs) after HT treatment (Figure 5B and Table S2). Among the 1,447 genes, 867 genes were specifically regulated at 3 weeks, while 4,991 out of 5,571 genes were specifically regulated at 8 weeks. A total of 580 genes were co-regulated at both 3 and 8 weeks at high temperatures. Heatmap analysis of these co-regulated genes showed similar expression patterns at 3 and 8 weeks (Figure 5C). Because our purpose was to identify genes involved in temperature-responsive tuberization, we performed gene ontology (GO) analysis using the gene groups regulated by 3- or 8-week treatment at high temperatures. Genes involved in biological functions, including ethylene response, defense, redox homeostasis, and endopeptidase activity were analyzed as significantly overrepresented GO terms from the 3-week gene groups (Figure 5D). Meanwhile, genes involved in more diverse biological functions, including flower development, cell division, and cell wall metabolism, were analyzed from the 8-week gene groups (Figure 5E).

Transcriptome analysis of potato plants exposed to high temperature was also performed in the previous report (Hastilestari et al., 2018). Thus, we compared our results with the previously reported transcriptome data to find common heat-regulated genes from the independent research. Among the 1,447 DEGs at 3 weeks, 182 DEGs are listed in the previous results (Table S3A). About 62% (113 DEGs) showed similar expression patterns in our and the previous data. GO analysis using the 113 DEGs showed that genes involved in heat, high light, and reactive oxygen species (ROS) responses were regulated by high temperature (Figure S6A), suggesting that stress responses are activated by heat at 3 weeks. Among the 5,571 DEGs at 8 weeks, 673 DEGs are listed in the previous results (Table S3B). The percentage of DEGs showing similar expression patterns was only about 41% (279 DEGs), possibly because the transcriptome analysis was performed using the plants that were exposed to heat for 2 weeks in the previous reports. GO analysis using the 279 DEGs showed that genes involved in photosynthesis as well as the stress responses were regulated by high temperature (Figure S6B).

Previous studies have shown that potato homologs of *Arabidopsis* genes involved in flowering-time regulation are related to tuberization (Abelenda et al., 2016; Campbell et al., 2008; Navarro et al., 2011). In addition, ethylene and GAs have been suggested as phytohormones involved in potato tuber formation and tuber swelling (Mingo-Castel et al., 1976; Roumeliotis et al., 2012; Xu et al., 1998). Our GO analysis showed that genes involved in ethylene response and flowering time were responsive to high temperatures at 3 and 8 weeks, respectively (Figures 5D and 5E). On the basis of the previous reports and our data, we selected genes that have GO terms related to flowering time, GA

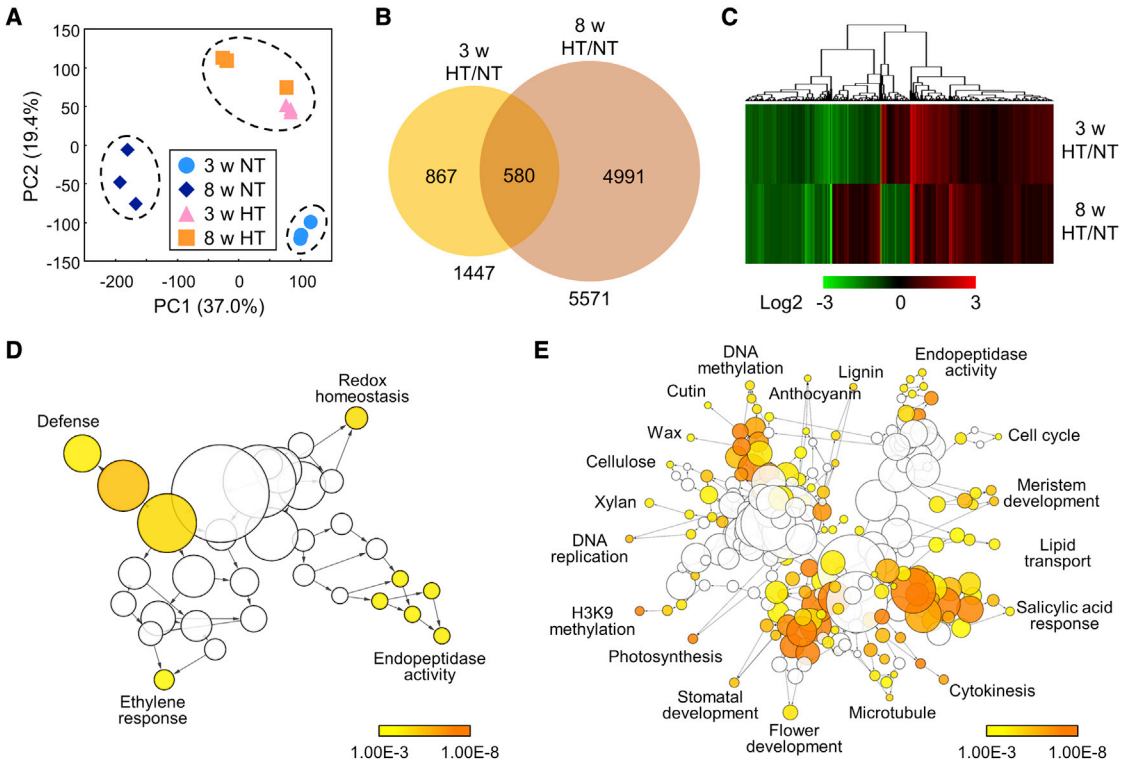

Figure 5. Transcriptome analysis at different stages and temperatures during tuberization

(A) PCA plot of transcriptome data. X axis represents PC1, which explains 37.0%, and Y axis represents PC2, which explains 19.4% of the variability for gene expression. Three biological replicates are shown by the same color and shape spot. Samples belonging to the similar place are grouped by circles. NT, normal temperature (22/16°C); HT, high temperature (35/29°C).

(B) Venn diagram shows the number of DEGs by HT treatment for 3 or 8 weeks. Genes with $\log_2$ fold change greater than 1.5-fold and p value < 0.01 were considered DEGs.

(C) Heatmap of DEGs at both 3 and 8 weeks. Red denotes up-regulated and green denotes down-regulated genes at HT compared with those at NT. Scale bar indicates fold changes ($\log_2$ value).

(D and E) GO analysis of DEGs at 3 (D) or 8 (E) weeks after HT treatment. Colored nodes in the network diagram represent significantly overrepresented GO terms (Benjamini-Hochberg-corrected p < 0.001). Scale bars indicate p values.

biosynthesis, and ethylene responses from the DEGs. Expression of most genes involved in flowering time and GA biosynthesis exhibited a higher difference at 8 weeks between NT and HT than at 3 weeks, while expression of ethylene-responsive genes showed a higher difference at 3 weeks (Figure 6A). To confirm the RNA-sequencing results, we analyzed expression of candidate genes possibly involved in thermo-regulation of tuberization. As analyzed by RNA-sequencing, expression of *CENTRORADIALIS* (*StCEN1*) and *StGA2ox1-1* genes was significantly decreased by HT at 8 weeks (Figure 6B). The expression of *StCOL1* was significantly increased by HT at both 3 and 8 weeks. Expression of the *ETHYLENE RESPONSE FACTOR 5-3* (*StERF5-3*) was increased by HT; however, the difference between NT and HT was statistically significant only at 3 weeks due to high variability. Although *StSP5G* was not detected in DEGs from the RNA-sequencing results, we analyzed its expression because *StSP5G* has been reported as a key negative regulator of *StSP6A* during photoperiod-dependent tuberization (Abelenda et al., 2016). However, expression of *StSP5G* was not significantly altered by HT treatment (Figure 6B), suggesting that the temperature-dependent regulatory mechanisms of tuberization are different from those related to photoperiod. Expression of these genes was also analyzed in stolons, but expression patterns were different between leaves and stolons (Figure 6C). All together, our data indicated that different regulatory mechanisms function at the specific period of tuberization under high-temperature conditions, which delays tuber development and decreases tuber yield.

DISCUSSION

In this study, we performed time course analysis of tuberization to examine how plants respond differently at different stages of tuberization under high-temperature conditions. On the basis of our data, we defined three stages of tuberization: initiation, early stage, and late stage. Regulation of *StSP6A* transcript levels in leaves plays a key role in temperature-dependent tuber formation. At the initiation stage, expression of *StSP6A* starts to increase, but the transcript levels are low due to transcriptional suppression (Figure S7). In this stage, expression of *StSP6A* is similar at NT and HT, but tuberization starts only at NT. These findings suggest that another negative regulator is activated by

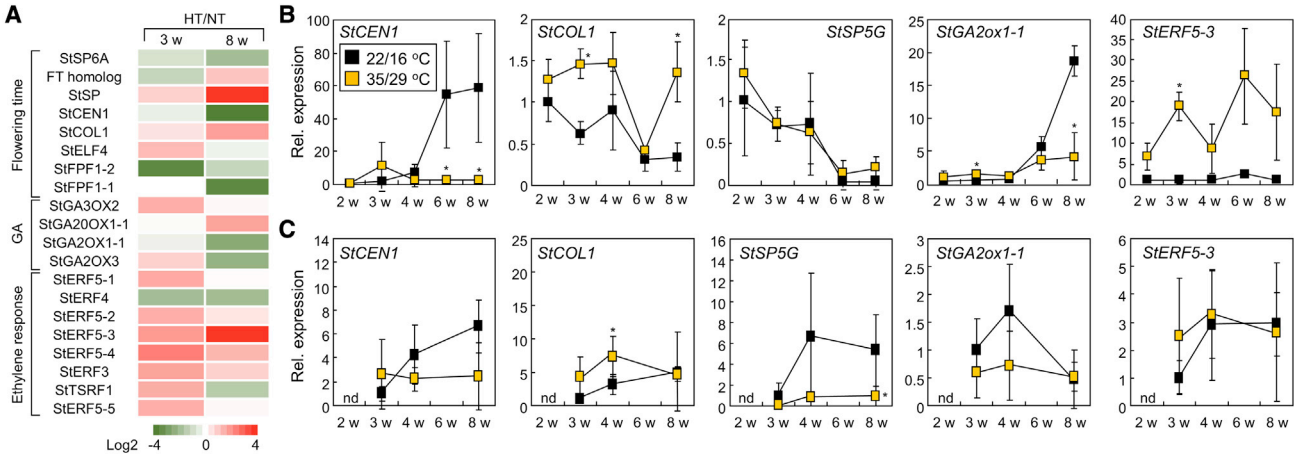

Figure 6. Expression of the selected genes from the transcriptome analysis

(A) Expression of the candidate genes for the temperature-dependent tuberization. Genes involved in flowering time, GA biosynthesis, and ethylene responses were selected based on GO annotation among DEGs analyzed in Figure 5. Fold changes in gene expressions were visualized using heatmap. The color scale represents $\log_2$ fold changes.

(B and C) Time course expression of the selected genes at different temperatures. WT plants were grown as described in Figure 1 for the indicated time points. Expression of genes was analyzed in leaves (B) and stolons (C). Three to five replicates were averaged and statistically analyzed (t test, *$p < 0.05$). Error bars indicate ± SD.

high temperature to suppress tuber formation. At the early stage, transcriptional repression of *StSP6A* is partially released; thus, the tuberization is facilitated at NT. However, negative regulators including SES post-transcriptionally repress the *StSP6A* levels at high temperatures, resulting in delayed tuberization. At the late stage, transcriptional repression is not released at high temperatures, which causes suppression of tuber formation.

Transgenic plants that show about 15,000-fold more expression of a codon-optimized version of *StSP6A* exhibit reduced shoot weight but increased tuber yield, resulting in a high tuber/shoot ratio (Lehretz et al., 2019). However, in our data, overexpression of a native *StSP6A* did not increase the tuber/shoot ratio at NT (Figure S4). The difference between the previous report and our data is due to expression levels of *StSP6A* in *35S*:*StSP6A* transgenic plants. Overexpression of the native *StSP6A* increased the expression about 100- to 200-fold when the plantlets were grown for 1 week (Figure 3C). However, the intrinsic expression of *StSP6A* increased more than 200- and 400-fold when the plantlets were grown for 5 and 8 weeks, respectively (Figure 3A), masking the ectopic expression. Therefore, *StSP6A* transcript levels were not largely increased by the overexpression at 5 and 8 weeks under NT conditions (Figure 4A), resulting in similar tuber/shoot ratio between the wild-type and the *35S*:*StSP6A* transgenic plants. On the other hand, we could observe the effects of the ectopic overexpression of *StSP6A* at high temperature, because the intrinsic transcript levels of *StSP6A* were largely suppressed at HT (Figure 3A). Overexpression of *StSP6A* partially and completely restored the thermo-suppressed *StSP6A* transcript levels at 5 and 8 weeks, respectively. However, increased *StSP6A* expression improved only tuber formation but not tuber yield (Figure 3G); thus, the tuber/shoot ratio was not noticeably altered by *StSP6A* overexpression (Figure S4). Notably, effects of the *StSP6A* overexpression were different in stolons and tubers. The *StSP6A* transcript levels were not enhanced in the *35S*:*StSP6A* stolons at both NT and HT (Figure 4B). In tubers, HT increased the *StSP6A* expression and the *StSP6A* overexpression upregulated the transcript levels at both NT and HT (Figure 4C). These results indicate that tissue-specific regulatory mechanisms exist to control the *StSP6A* transcript levels.

We tried to identify unknown factors that suppress tuber formation at the initiation stage under high-temperature conditions. RNA-sequencing at 3 and 8 weeks showed that expression of *StCOL1* and *StERF5-3* was upregulated by HT at the initiation stage (~3 weeks; Figure 6). The StCOL1-StSP5G regulatory module has been identified in photoperiod-dependent tuberization. Expression of *StCOL1* peaks at dawn, and suppression of *StCOL1* results in decreased expression of *StSP5G* (Abelenda et al., 2016). StCOL1 directly activates StSP5G, which negatively regulates *StSP6A* expression. Together with our expression analysis, StCOL1 might be involved in suppression of tuberization at high temperatures. However, unlike *StCOL1*, expression of *StSP5G* was not altered by high temperature in our study. Therefore, it seems that photoperiod- and temperature-dependent tuberization pathways have different regulatory mechanisms.

The ERF transcription factors upregulate the expression of ethylene-responsive genes (Hu et al., 2008). Therefore, StERF5-3 possibly mediates ethylene signaling in potatoes to suppress tuberization at high temperatures. Consistent with this idea, the PCA plot from our transcriptome data showed that the samples at NT exhibited different patterns between 3 and 8 weeks, but those at HT exhibited similar patterns. At high temperatures, it is likely that early accumulation of the aging hormone ethylene might accelerate developmental processes (Schaller, 2012). In addition, previous reports have shown that ethylene accumulates in plants exposed to elevated

temperatures (Huberman et al., 1997). Because ethylene treatment delays tuber formation in potatoes (Mingo-Castel et al., 1976), StERF5-3-mediated ethylene signaling could be a candidate that regulates potato tuberization at high temperatures. Studies on the function of StERF5-3 will provide clues for determining the role of ethylene in temperature-dependent tuber development.

GAs are important phytohormones that regulate tuber initiation and swelling (Martinez-Garcia et al., 2002; Shi et al., 2016; Xu et al., 1998). Overexpression of *StGA20ox1*, which mediates the biosynthesis of bioactive GAs, delays tuberization; contrastingly, overexpression of *StGA2ox1*, which inactivates GAs, accelerates tuber development (Carrera et al., 2000; Kloosterman et al., 2007). In addition, bioactive GA levels rapidly decrease in stolon tips during tuber swelling (Roumeliotis et al., 2012). These results indicate that GA is a negative regulator of potato tuberization. Our data showed that expression of *StGA2ox1-1* at 8 weeks under NT conditions was significantly higher than that under HT conditions (Figure 6B). These results suggest that high temperature controls GA levels in leaves. Analyses of bioactive GA content in stolons and temperature-dependent tuber development using *StGA2ox1*-overexpression plants are required to uncover the role of GAs in tuberization at high temperatures.

Complete recovery of the *StSP6A* transcript levels in leaves improved only the tuber number but not the tuber yield at the late stage under HT conditions (Figures 3G and 4A). A previous report has shown that co-overexpression of *StSP6A* with *Arabidopsis* hexokinase 1 (*AtHXK1*) enhances tuber yield under heat stress conditions (Lehretz et al., 2021a). Because AtHXK1 decreases stomatal conductance, water transpiration is significantly reduced in the co-overexpression plants. Therefore, it is possible that other molecular factors involved in water use efficiency in addition to StSP6A would be required to completely restore tuberization at high temperatures.

Based on our observations of sugar contents in the leaves (Figures 4G and 4H), the decreased yield might be due to suppressed sugar transport from leaves to tubers. Inhibition of the sugar transport by suppressing sugars will eventually be exported transporter 11 (StSWEET11) results in reduced tuber yield with accumulation of sugars in leaves (Abelenda et al., 2019). Similarly, we observed that sugar contents were highly increased in leaves from the plants grown at HT (Figures 4G and 4H). These data suggest that high temperature blocks source-to-sink sugar transport to inhibit tuber growth. Previous reports reveal that StSP6A inhibits sucrose leakage from the phloem into the neighboring cells (Abelenda et al., 2019; Lehretz et al., 2021b). However, our data show that *StSP6A* overexpression did not largely affect increase of sugar contents at HT (Figures 4G and 4H), suggesting that there are StSP6A-independent mechanisms on heat-induced sugar accumulation in the leaves. Future studies on the sugar transporters at different temperatures will provide clues for thermo-regulation of tuber growth in potato.

Although we identified a total of 1,447 and 5,571 thermo-responsive genes at 3 and 8 weeks, respectively, only a small number of genes are commonly listed in our and the previous transcriptome data. This is possibly due to different cultivars and experimental conditions used in each study. Despite the differences, we found that the commonly identified DEGs are involved in high light, heat, and ROS responses at both 3 and 8 weeks after high-temperature treatment (Figures S6A and S6B). Because high light and heat stresses cause ROS accumulation in plants (Apel and Hirt, 2004; Suzuki and Mittler, 2006), exposure to high temperature would trigger oxidative stress in potato at both early and late stages of tuberization. Notably, expression of the photosynthesis-related genes including Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) activase (PGSC0003DMT400028767) was changed at 8 weeks under HT conditions (Figure S6B and Table S3B). Because heat stress deactivates Rubisco and suppresses photosynthesis (Salvucci and Crafts-Brandner, 2004), it seems that the photosynthesis-related genes are controlled to compensate the reduced photosynthesis at high temperature.

Although we analyzed *StSP6A* transcript levels and other high-temperature-responsive genes mainly via RNA-sequencing during tuberization, these data cannot fully explain the thermo-regulation of tuber development in potato. Expression of the *StSP6A* is mainly controlled in the leaves, but the protein moves to the stolon where post-translational regulation occurs (Abelenda et al., 2016). StSP6A interacts with diverse proteins to control tuberization. Complex formation of StSP6A with 14-3-3 and FD-like 1 (FDL1) proteins is critical for tuberization; thus, effects of *StSP6A* overexpression on inducing tuber formation are largely reduced when a key amino acid for the protein interaction is mutated (Teo et al., 2017). In addition, overexpression of a competitive inhibitor of StSP6A-FDL1 interaction decreases tuber yield (Zhang et al., 2020). StSP6A also interacts with a sucrose transporter, StSWEET11, to block sucrose leakage from the tuber (Abelenda et al., 2019). Together with the previous reports and our data, post-translational regulation of StSP6A would also be important for thermo-regulation of tuberization. Screening StSP6A-interacting proteins under different temperature conditions will be necessary to understand detailed molecular mechanisms on the temperature-regulated tuber development.

In addition to StSP6A, there are additional molecular factors regulating tuberization in potato. The BEL1-like protein StBEL5 is a transcription factor that binds to the tandem TGAC motif contained within tuberization-related genes including *StSP6A* (Sharma et al., 2016; Kondhare et al., 2019; Chen et al., 2004). Transcripts of *StBEL5* act as a mobile signal to induce tuber formation (Banerjee et al., 2006). Our transcriptome analysis showed that expression of *StBEL5* (PGSC0003DMT400050527) was largely suppressed under HT conditions at 8 weeks (Table S2B), raising possibility that temperature-dependent regulation of *StBEL5* is important for tuberization.

Plant microRNAs (miRNAs, miR) are known as another key player in tuber development. Potato miR172 is a phloem-mobile small RNA whose abundance is elevated under SD (Martin et al., 2009). miR172 promotes tuber formation, possibly through upregulation of *StBEL5* expression. On the contrary, potato miR156 suppresses tuber development (Bhogale et al., 2014). The miR156 overexpression reduces miR172 levels and *StSP6A* expression, possibly through suppression of SQUAMOSA PROMOTER BINDING LIKE transcription factors (Bhogale et al.,

2014; Natarajan et al., 2017). Because our transcriptome analysis showed that a number of genes are regulated by high temperature, it is possible that miRNAs including miR172 and miR156 are involved in temperature-dependent regulation of tuberization. Analysis of miRNA abundance at different temperatures through small-RNA-sequencing will provide clues on the role of miRNAs in these responses.

Limitations of the study

Although we identified that regulation of *StSP6A* transcript levels is important for tuber formation, it is still unknown which molecular factors are responsible for decreased tuber yield at high temperature. Our data showed that recovery of the decreased *StSP6A* expression at HT is sufficient for restoring tuber numbers but is not highly effective in increasing tuber yield per plant (Figures 3F, 3G, and 4A). These results indicate that there are different molecular mechanisms for the regulation of tuber formation and tuber swelling at high temperature. Analysis of sugar contents in the leaves provided some clues for heat regulation of tuber yield. In the leaves of heat-exposed plants, sugars including sucrose, glucose, and fructose were highly accumulated at both early and late stages of tuberization (Figures 4G and 4H). In combination with the previous reports that sugar transporters play an important role in the regulation of tuber yield (Abelenda et al., 2019), genes involved in source-to-sink sugar transport might be regulated by high temperature. We tried to identify novel genes whose functions are related to the regulatory pathways on the heat-suppressed tuber yield by transcriptome analysis (Figure 5). However, too many genes were responsive to high temperature, which made it difficult for us to find key genes for these processes. In summary, our primary limitation is lack of finding key players in addition to StSP6A during heat-suppressed tuberization. Thus, we cannot fully explain how potato plants control tuber formation and swelling under high-temperature conditions.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2022.110579.

ACKNOWLEDGMENTS

We thank Dr. Hyunjun Ko for analyzing sugar contents. This work was supported by the Basic Research Program provided by the National Research Foundation of Korea (NRF-2019R1C1C1002045 and -2019R1A2C1003798), the KRIBB Research Initiative Program (KGM5372221) and the New Breeding Technologies Development Program (Project No. PJ0165302022).

AUTHOR CONTRIBUTIONS

H.-J.L., H.-S.K., and J.-S.P. conceived and designed the experiments. J.-S.P. performed most of the experiments. J.-S.P., S.-J.P., and K.-B.M. harvested tubers and stolons for measuring yield and size. J.-S.P. and S.-J.P. extracted total RNA from the tubers and stolons. S.-Y.K. and A.-Y.S. performed the RNA-sequencing. H.-J.L., S.-Y.K., and J.-S.P. analyzed RNA-sequencing data. J.-S.P., H.-S.K., and H.-J.L. prepared the manuscript with the contributions of S.-J.P., K.-B.M., J.M.P., H.S.C., S.U.P., and J.-H.J.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: October 12, 2020
Revised: December 14, 2021
Accepted: March 4, 2022
Published: March 29, 2022

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
E. coli strain DH5α	Enzynomics	CP011
A. tumefaciens strain GV3101	Widely distributed	N/A
Chemicals, peptides, and recombinant proteins		
Murashige & Skoog medium including vitamins	Duchefa	Cat#M0222.0050
Plant agar	Duchefa	Cat#P1001.1000
Carbenecillin Disodium	Duchefa	Cat#C0109.0025
Zeatin	LPS solution	Cat#ZEAT01
a-Naphthaleneacetic Acid	Sigma-Aldrich	Cat#N-0375
Phosphinothricin	GoldBio	Cat#P-165-1
Gibberellic Acid	Duchefa, Haarlem	Cat#G0907
Critical commercial assays		
RNeasy Plant Mini Kit	QIAGEN	Cat#74904
miRNeasy Mini Kit	QIAGEN	Cat No./ID: 217004
TOPscript cDNA Synthesis Kit	Enzynomics	Cat#EZ005M
miScript Plant RT Kit	QIAGEN	Cat No./ID: 218761
TOPreal™ qPCR 2X PreMIX	Enzynomics	Cat#RT005M
miScript SYBR Green PCR Kit	QIAGEN	Cat No./ID: 218073
Trizol reagent	MRC	Cat#TR118
HEPES	Gibco	Cat#15630-080
Deposited data		
RNA-sequencing data using wild type plants	This paper	GEO: GSE158644
Experimental models: Organisms/strains		
Solanum tuberosum cv. Desiree	Widely available	N/A
35S::StSP6A	This study	N/A
Oligonucleotides		
Stu_snoR12_1 miScript Primer Assay	QIAGEN	MS00064253
Oligonucleotides are described in Table S1	N/A	N/A
Recombinant DNA		
pEarleyGate202-35S::StSP6A	This study	N/A
pDONR221	ThermoFisher	Cat#12536017
pEarleyGate202	TAIR	Stock#CD3-688
Software and algorithms		
Excel	Microsoft	N/A
R Studio	https://rstudio.com/	N/A
CLC Genomics Workbench	QIAGEN	N/A
Cytoscape	https://cytoscape.org/	N/A
Java TreeView	https://sourceforge.net/projects/jtreeview/	N/A
Other		
BioRad CFX Connect Real-Time System	Bio-Rad	N/A
UV-visible recording spectrophotometer	SHIMADZU	N/A
Plant growth chamber	JSR	N/A
Hiseq 4000 platform	Illumina	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hyo-Jun Lee (hyojunlee@kribb.re.kr).

Materials availability

All materials are available upon request from the lead contact.

Data and code availability

- RNA sequencing data have been deposited in Gene Expression Omnibus with the accession number GEO: GSE158644 and are publicly available as of the date of publication.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Potato genotypes and growth conditions

The *Solanum tuberosum* L. cultivar Desiree was used in this study. The potato transgenic plants overexpressing *StSP6A* were generated in this study. The Desiree and transgenic plants were propagated in petri dish with a diameter of 11.5 cm and a height of 2 cm containing MS medium (Duchefa) supplemented with 30 g/L sucrose and 8 g/L plant agar (Duchefa). Plantlets were grown under 16 h light/8 h dark conditions at constant temperature of 24°C and light intensity of 100 μmol m^{-2} s^{-1}. To prepare the plantlets for tuberization, lateral buds were induced from the stem nodes in MS-agar plates for 2 weeks. The lateral buds were then excised and incubated in MS-agar plates for 4 weeks. Apical buds were subcultured every 2 weeks. To synchronize growth stages, apical buds were incubated in MS-agar plates for an additional week after the final subculture. Plantlets were acclimated for 1 day in water-containing plates at 24°C before they were transferred to the pots. Plantlets were then grown in the pots whose size was 11 cm in diameter and 10 cm in height at different temperatures indicated in the text without acclimation. Growth conditions of plantlets in soil are as follows: 12 h light/12 h dark; light intensity of 153 μmol m^{-2} s^{-1}.

METHOD DETAILS

Measurement of tubers and stolons

Potato plants were grown at the indicated temperatures for 5 and 9 weeks in soil. For measuring tuber yield, tuber number, stolon yield, and stolon number, tubers with width of 3 mm or more and stolons with length of 10 mm or more were analyzed. Yield of tuber and stolon means average value of total fresh weights harvested in each plant. For wild type, independent experiments were performed for 4 times. For *35S:StSP6A-1* and *-2*, experiments were performed for 3 times. For *35S:StSP6A-6*, a single experiment was performed. Four to five independent plants were used for each experiment. Measurement data were statistically analyzed using either a Tukey's test or a Student's *t*-test. Tukey's test was performed using R Studio software (https://rstudio.com/). Percentage of tuber numbers within the specific range of fresh weight or width among the total number of tubers was calculated and visualized using heatmap. Heatmap was visualized with Java TreeView software (Palaniswamy et al., 2006).

Generation of transgenic plants

For producing the potato plants overexpressing *StSP6A* gene, the 522 open reading frame sequences of PGSC0003DMT400060057 were amplified by PCR from cv. Desiree cDNA. Primer sequences are given in Table S1. The PCR products were subcloned into the pEG202 binary vector containing the CaMV 35S promoter using the gateway cloning system (Hartley et al., 2000). The constructs were introduced into cv. Desiree using *Agrobacterium tumefaciens* strain GV3101. The *A. tumefaciens* were grown up to OD_{600} of 0.5, and then cell cultures and potato leaf discs were co-cultivated for 20 min. Transgenic plants were selected in MS-agar plates containing 3 mg/L phosphinothricin (GoldBio), 30 g/L sucrose, 4 g/L plant agar, 2 mg/L Zeatin (LPS), 0.01 mg/L α-Naphthaleneacetic acid (Sigma-Aldrich), 0.1 mg/L GA_3 (Duchefa), and 500 mg/L Carbenicillin (Duchefa).

RNA isolation

For gene expression analysis, the independent potato plants were grown as conditions stated in the text. Upper 5 leaves and total stolons were harvested at zeitgeber time 5 (ZT5) for RNA isolation. Total RNA were extracted from 50 mg of frozen ground tissues using RNeasy Plant Mini kit (QIAGEN). For microRNA analysis, 50 mg of frozen ground leaves were used to isolate small RNAs using miRNeasy Mini kit (QIAGEN).

Reverse transcription and quantitative real-time PCR

For cDNA synthesis, RNA quantity and quality were measured from NanoDrop2000 spectrophotometer (Thermo Scientific) and 1 μg of RNA was used to synthesize cDNA using TOPscript™ cDNA Synthesis kit (Enzynomics). For cDNA synthesis of miRNA, 1 μg of small RNAs isolated as described above was used to synthesize cDNA using miScript Plant RT kit (QIAGEN).

Gene expression was analyzed by qPCR using TOPreal™ qPCR 2X PreMIX (Enzynomics) and CFX Connect Real-Time System (BIO-RAD). Expression of the miRNA was analyzed using miScript SYBR Green PCR kit (QIAGEN). Primers used in this study are described in Table S1.

Measurement of sugar contents

Potato leaves were harvested at 5 and 9 weeks. For sugar extraction, 500 mg of grinded plant materials was mixed with 500 μL of extraction solution containing 80% ethanol and 10 mM HEPES (Gibco) and then incubated at 80°C for 2 h. The supernatant was evaporated in a SpeedVac (Savant) vacuum, and then dissolved in 200 μL of distilled water. Sucrose, glucose, and fructose contents were measured using an Agilent Technologies 1200 HPLC system (Palo Alto).

RNA-sequencing

Leaves of the potatoes grown for 3 and 8 weeks at NT and HT were harvested for total RNA extraction. Total RNA was extracted from 100 mg of ground leaves using the Trizol reagent (MRC) following the manufacturer's instructions. Five μg of each RNA sample were generated for a strand-specific library as previously described (Zhong et al., 2011). The library was containing inserts of 150–200 bp. For RNA sequencing, paired-end 150 nt sequencing was performed using a HiSeq 4000 platform (Illumina, USA) at Microgen (Korea). The sequencing data were aligned to PGSC_DM_v3.4 transcripts database from potato genomic resource (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml).

For PCA plot and DEG analysis, raw RNA-sequencing data were processed using CLC Genomics Workbench (Insilicogen). Genes with p value < 0.01 and log2 fold change > 1.5 were regarded as DEGs. Expression of genes regulated by high temperature at both 3 and 8 weeks were visualized by heatmap using R software (https://www.r-project.org/). For GO analysis, functional annotation of potato genes was employed from the previous study (Amar et al., 2014). GO analysis was performed using Biological Networks Gene Ontology tool (BiNGO) with Benjamini-Hochberg-corrected $p < 0.001$. The network diagram was constructed using Cytoscape software (https://cytoscape.org/) and displays significantly overrepresented GO terms.

Gene annotation

Sequences of genes encoding StSP6A (PGSC0003DMT400060057), StCEN1 (PGSC0003DMT400037143), StCOL1 (PGSC0003DMT400026065), StSP5G (Sotub05g026730.1.1), StGA2ox1-1 (PGSC0003DMT400054348), and StERF5-3 (PGSC0003DMT400034490) were employed from the potato genome database in Spud DB potato genomic resources (http://solanaceae.plantbiology.msu.edu/).

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantifications are displayed in the graph as the mean ± standard deviation derived from at least three independent replicates. In box plots, each box extends from the first quartile to the third quartile values of the data, and center lines show median values. Whiskers show maximum and minimum values of the data. For phenotype analysis for potato tubers and stolons of wild type plants, 5 independent replicates were used. For phenotype analysis of wild type and *35S:StSP6A* plants, 1 to 4 independent experiments with 4 ∼ 5 replicates for each experiment were performed. The detailed number of replicates for all data were described in the figure legend. Statistical significance between two groups was determined by 2-tailed Student's *t*-test (*$p < 0.05$) using Microsoft Excel 2016. When more than two groups were compared, one-way ANOVA with Tukey's post-hoc test was performed using R Studio software and the statistical significance was marked as different letters ($p < 0.05$). No methods were used to evaluate whether the data met assumptions of the statistical approach.