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TITLE

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Running title: Tuberization signal represses potato flower development

HIGHLIGHT

For the first time it is shown that the tuberization signal StSP6A not only induces tuberization, but also represses flower bud development in potato

ABSTRACT

Potato (*Solanum tuberosum* L.) can reproduce sexually through flowering and asexually through tuberization. While tuberization has been thoroughly studied, little research has been done on potato flowering. Flower bud development in the strictly short-day tuberizing *S. tuberosum* group Andigena is impaired under short-day conditions. This impaired development may indicate that tuberization negatively influences flowering. The aim of this research was to determine how tuberization affects flower bud development. To find out whether the absence of tubers improves flowering we prevented tuberization by: (1) grafting potato scions onto wild potato rootstocks, which were unable to form tubers; (2) removing stolons, the underground structures on which tubers form; (3) using plants that were silenced in the tuberization signal *StSP6A*. Additionally, transgenic plants with increased *StSP6A* expression were used to determine if flower bud development was impaired. The absence of a tuber-sink alone did not accelerate flower bud development, nor did it allow more plants to reach anthesis (open flowering stage) or have more open flowers. Interestingly, reducing *StSP6A* expression improved flower bud development, and increasing expression impaired it. Our results show that flower bud development in potato is repressed by the tuberization signal StSP6A, and not by competition with the underground tuber-sink.

Keywords: day length, grafting, potato flowering, stolons, StSP6A, tuberization

INTRODUCTION

Potato (*Solanum tuberosum* L.*)* is the third largest crop for human consumption worldwide and due to its high nutritional value and low production costs, consumption is most certainly expected to increase (International Potato Center, 2016; Zaheer and Akhtar, 2016). Potato plants are able to reproduce both sexually, through flowers, and asexually through the formation of tubers. Although both reproduction methods are present in the plant, most research has been done on tuberization. Commercial potato production mainly uses "seed tubers" and not "true seeds" to propagate plants. Asexual reproduction is used for propagation because potato plants are tetraploid and highly heterozygous. Incorporation of a *Sli* gene allows for self-fertilization of diploid potato lines, which makes the generation of homozygous lines possible (Lindhout *et al*., 2011). These developments have made hybrid breeding in potato possible and thereby also the use of true potato seeds as starting material. Hybrid breeding of potato will enable breeders to specifically select for desired traits in new varieties and develop these varieties much faster than in traditional potato breeding (Lindhout *et al*., 2011). The developments in potato breeding and propagation require the understanding of not only tuberization, but also potato flowering.

Whether a potato plant starts to tuberize or flower, depends strongly on environmental cues (Ewing and Struik, 1992; Almekinders and Struik, 1996). Potato tuberization is strongly influenced by day length and is induced under short-day conditions (Batutis and Ewing, 1982). Modern varieties are no longer dependent on short days to tuberize, as breeders have selected against this trait. Nevertheless, the photoperiodic mechanism controlling tuberization remains conserved in all potato plants (Kloosterman *et al*., 2013). As potato tuberization has been intensively studied, we have a good understanding of the molecular regulation behind this process (Abelenda *et al.* 2011; Navarro *et al.*, 2011; González-Schain *et al.* 2012; Navarro *et al.*, 2015). The photoperiodic regulation of tuberization strongly resembles the photoperiodic control of flowering time in the model plant *Arabidopsis thaliana* and other plants (Tsuji *et al.,* 2011; Andrés and Coupland, 2012; Fu *et al.*, 2014). *SELF-PRUNING 6A* (*StSP6A*) was identified as a potato homologue of the flowering signal *FLOWERING LOCUS T* (*FT*) in *A. thaliana* and instead of inducing the flower transition, StSP6A induces tuber formation in potato (Potato Genome Sequencing Consortium, 2011; Navarro *et al.*, 2011). After *StSP6A* is expressed in the leaves, the mobile StSP6A protein moves through the plant to underground stems, called stolons, where it induces tuberization. The cascade of events leading to short-day

dependent expression of *StSP6A* mRNA has also been revealed. This control includes the genes *CYCLING DOF FACTOR* (*StCDF1*) and *CONSTANS* (*StCO*) (Kloosterman *et al*., 2013), which are also involved in photoperiodic control of flowering in *A. thaliana*. In potato StCDF1 downregulates *StCO*, which in turn induces *SELF-PRUNING 5G* (*StSP5G),* a repressor of *StSP6A* (Kloosterman *et al*., 2013; Abelenda *et al.*, 2016). Within the Solanaceae, the *FT* family has undergone a large expansion and another homologue of *FT* called *SELF-PRUNING 3D* (*StSP3D*) was found in potato and was proposed to control the flower transition (Potato Genome Sequencing Consortium, 2011; Navarro *et al.*, 2011). However, how this regulation takes place remains to be elucidated.

Although some research has been performed on potato flowering, ambiguity remains concerning the environmental effect on flower transition and whether this is a long-day, short-day or day-neutral processes (Jones and Borthwick, 1938; Almekinders and Struik, 1994; Navarro *et al.*, 2011; González-Schain *et al.*, 2012). Although little is known about the flower transition, it has been established that potato flower development is negatively affected in tuber inducing conditions like short days (Turner and Ewing, 1988; Rodríguez-Falcón *et al.*, 2006; Plantenga *et al*., 2016). Flower buds abort more frequently and less open flowers are formed. Failure of flower bud development in short days could be due to a direct photoperiod effect, but alternatively might be the result of a negative effect exerted by tuberization. Tubers are strong assimilate sinks (Sweetlove *et al.,* 1998) and may leave insufficient assimilates to support flowering (Almekinders and Struik, 1996). However, previous studies do not agree whether or not flowering competes with tuberization (Krantz, 1939; Thijn, 1954; Jessup, 1958; Krauss and Marschner, 1984; Pallais, 1987).

Here we confirm that while the flower transition occurs independently of photoperiods, later stages of flower bud development are impaired under short-day conditions which induce tuberization. Specifically, we investigated whether flower bud development is impeded by competition for assimilates between flowering and tuberization or by the tuberization signal StSP6A. We performed experiments where we prevented tuberization in three different ways; (1) by grafting potato scions onto wild potato rootstocks, that were unable to form tubers; (2) by removing stolons, the structures on which tubers form; (3) by using transgenic plants that were silenced in the tuberization signal *StSP6A* (Fig. 1). Finally we demonstrated how increased *StSP6A* expression affected flower bud development in long days. Together, our experiments show that the tuberization signal StSP6A inhibits flower bud development and only the repression of this signal improves flower bud development.

MATERIALS AND METHODS

Plant materials

Solanum tuberosum group Andigena (*S. andigena*), *Solanum tuberosum* CE3027 and *Solanum tuberosum* CE3130 were used. *S. andigena* is a tetraploid, obligatory short-day plant for tuberization. CE3027 and CE3130 are progeny plants from a mapping population that segregates for timing of tuberization (Kloosterman *et al*., 2013), where CE3027 tuberizes early in short days and late in long days, and CE3130 tuberizes early under both short and long days. These lines were used because they can produce open flowers in our climate chamber conditions, as opposed to *S. andigena*. All genotypes were propagated *in vitro* and maintained in tissue culture in MS20 medium (Murashige and Skoog, 1962). Additionally, two wild *Solanum* species that are unable to tuberize were used: *Solanum etuberosum* (CGN17714) and *Solanum palustre* (CGN18241) (CGN seedbank, Wageningen, Netherlands). Seeds of these species were disinfected in 2.7% NaOCl for 30 minutes, soaked in 700ppm gibberellic acid (GA_3) for 24 hours in the dark and sown on MS20. Finally, two *StSP6A* silenced lines in a *S. andigena* background (*StSP6A RNAi #1* and *StSP6A RNAi #13*) and two *StCDF1* overexpressing lines in a CE3027 background (*35S::StCDF1#3 and 35S::StCDF1 #4)* were used.

Plant transformation

In order to generate these lines, *StSP6A* coding regions were PCR amplified from *Solanum tuberosum* group Andigena cDNA through Phusion High-Fidelity DNA Polymerase (Thermo Scientific™) using specific primers (RNAi6Afor 5'-CACCTACAAATACAAGCTTTGGAA-3' and RNAi6Arev 5'-CTCTATTTATTTATAACAT-3'). Then, cloned in pENTR™/D-TOPO® (Invitrogen) following manufacturer recommendations. The final *StSP6A RNAi* construct was generated using the *StSP6A* pENTR™/D-TOPO entry clone and further insertion by recombination with the LR clonase™ II enzyme (Invitrogen) into the pK7GWIWG2(II) vector (Karimi *et al.*, 2002). Transgenic plants bearing the *StSP6A RNAi* construct were generated by *Agrobacterium*-mediated transformation of *in vitro* internodes as described previously in Visser (1991) .

The StCDF1.1 coding region was also amplified with Phusion High-Fidelity DNA Polymerase (Thermo Scientific™) from *Solanum tuberosum* group Andigena cDNA (same primers as for RNAi) and cloned in pENTR™/D-TOPO® (Invitrogen) as previously described (Kloosterman

et al., 2013). Binary plasmids were obtained after LR clonase™ II enzyme (Invitrogen) reaction of StCDF1.1-pENTR™/D-TOPO® with the pK7WG2 plasmid, obtaining the 35S::StCDF1.1 plasmid (Karimi et al., 2002). In order to generate 35S::StCDF1 transgenic plants, *Agrobacterium*-mediated transformation of CE3027 internodes with both plasmids was performed as described in Visser (1991).

S. andigena StSP6A RNAi and CE3027 *35S::StCDF1* plantlets were propagated *in vitro* and grown with the other potato plants.

Growing conditions and measurements

Exp.1. Removing the tuber-sink: grafting onto a non-tuberizing rootstock

Two grafting experiments were performed in a greenhouse in short- and in long-day conditions. In short days CE3027 scions were grafted onto *S. etuberosum* and *S. palustre* rootstocks and vice versa. Also control grafts were made where scions were grafted onto rootstocks of their own genotype. In long days, nine grafting combinations were made between CE3027, CE3130 and *S. etuberosum*. CE3027 and CE3130 scions were grafted onto *S. etuberosum* rootstocks, *S. etuberosum* scions were grafted onto CE3027 and CE3130 rootstocks, and control grafts were made with scions and rootstocks from the same genotype. Additionally, *S. etuberosum* scions were grafted onto CE3027 and CE3130 rootstocks, which maintained their leaves, to ensure the production of the tuberization signal StSP6A (climate details in Supplementary Table S1). *In vitro* plantlets were transplanted to 5 L pots with a clay-peat mixture. Grafting was done with two-week old CE3027 and CE3130 plants and three-week old *S. etuberosum* and *S. palustre* plants. The stem was cut after the fourth leaf from the bottom. A splice-graft was made and the rootstock and scion were kept together with silicone grafting clips (Beekenkamp 1.5 mm and Simonetti 2.9 mm). Leaves were removed from the rootstock, unless indicated otherwise. Grafts were placed in a high humidity compartment until the grafting unions had set. The plants were manually watered and fertilized ($2g \cdot L^{-1}$, Osmocote Exact Standard 3-4M, Everris). Flowering and tuberization was determined once a week. Anthesis (opening flowering stage) of the primary stem and the maximum number of open flowers per plant were noted. Tuberization time was determined by carefully checking the stolon tip for swelling. Nine weeks after grafting, the tubers were harvested, oven-dried at 105ºC and weighed.

Exp. 2. Removing the tuber-sink: removing stolons

CE3027 plantlets were transplanted to 17cm Ø pots with a clay-peat mixture and placed in a climate chamber (details in Supplementary Table S1). Plants were grown in short days (8 hours light) under 200 or 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (photosynthetic photon flux density) light (SD200 and

SD400 respectively) and in long days (16 hours light) under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light (LD200). The high-light short day and low-light long day received the same daily light sum. In half of the plants in each light treatment, stolons were removed as soon as formed, resulting in six treatments in total. Light emitting diodes (LEDs) were used for the lighting (Philips GreenPower LED production module 120 cm DeepRed/White-2012). Light intensities were measured at the top of the plant canopy with a quantum sensor (LI-COR Biosciences, LI-190SB Quantum, LI-1400 data logger) and corrected by adjusting LED height every two weeks. Plants were rotated three times a week to ensure a homogenous light distribution. Side-shoots were removed. Water was given manually and liquid fertilizer was supplied once per week (EC 2.1 dS m^{-1}, pH 5.5; 1.2 mM NH_4^+, 7.2 mM K^+, 4.0 mM Ca^{2+}, 1.82 mM Mg^{2+}, 12.4 mM NO_3^-, 3.32 mM SO_4^{2-}, 10 mM P, 35 µM Fe^{3+}, 8.0 µM Mn^{2+}, 5.0 µM Zn^{2+}, 20 µM B, 0.5 µM Cu^{2+}, 0.5 µM MoO_4^{2-}). Plants were examined three times a week for stolons, flower bud appearance, anthesis, number of flowers and tuberization. A destructive harvest including fresh and dry weight measurements of tubers and shoot (aboveground stem, leaves and shoot apex) was done after eight weeks.

Exp. 3. Removing the tuberization signal: reducing StSP6A expression

Plants of *S. andigena* wild-type and two *StSP6A RNAi* lines (#1 and #13) were transplanted to 17cm Ø pots and placed in a climate chamber (details in Supplementary Table S1). In addition to the three light treatments used in Exp. 2, a long-day treatment of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LD400) was applied. Plants were grown and examined as in Exp. 2. Additionally, flower bud development was recorded (flower bud size was categorized from zero to five where zero was no flower bud and five was an open flower). This was done due to the bad flowering success of *S. andigena* and the low chances of reaching anthesis. A destructive harvest was performed after eight weeks of growing and included fresh and dry weight measurements of tubers and shoot.

Exp. 4. Removing the tuberization signal and tuber sink: reducing StSP6A expression and removing stolons

S. andigena wild-type and *StSP6A RNAi* #13 plants were transplanted to 17cm Ø pots and placed in a short-day chamber with 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light from fluorescent tubes (Philips; Master TL-D Reflex 58W/840 Coolwhite) (climate details in Supplementary Table S1). In half of the wild-type *S. andigena* plants, stolons were removed. Stolons were also removed in half of the *StSP6A RNAi* #13 plants to determine whether stolon removal affected plant growth in non-tuberizing plants. Plant growth control and determination of tuberization time and flower bud appearance were performed as in Exp. 2 and 3. Because flower bud size was only

categorized and not measured precisely in Exp. 3, flower bud development in Exp. 4 was determined by measuring the diameter of the biggest flower bud on each plant, three times a week.

Exp. 5. Increasing the tuberization signal: overexpressing StCDF1 in long days

An additional experiment was performed to confirm that StSP6A affected flower bud development. Instead of reducing *StSP6A* in short days, *StCDF1* overexpressing lines were used with upregulated *StSP6A* expression in long days. Eight wild-type CE3027, eight *35S::StCDF1#3* and eight *35S::StCDF1 #4* plantlets were transferred to 15 cm Ø pots and placed in a long-day chamber with 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light from fluorescent tubes (Philips; Master TL-D Reflex 58W/840 Coolwhite) (details climate in Supplementary Table S1). Plant growth control was performed as in Exp. 2, 3 and 4. Photographs of the shoot apex were taken after eight weeks of growing and anthesis was documented.

RNA analysis

StSP6A expression was analyzed to determine if the *StSP6A* silenced lines were indeed silenced in *StSP6A* and if the *StCDF1* overexpressing lines had upregulated *StSP6A.* Furthermore*, StSP3D* expression was analyzed to determine if the *StSP6A* silenced lines did not increase expression of the flowering signal *StSP3D*. Leaf samples of the plants in Exp. 3 were collected after five weeks, just before the first tuberization started. The fifth leaf from the top was sampled one hour after the lights went on. Leaves from three plants were collected, pooled into one sample and frozen in liquid nitrogen and stored at -80ºC. Leaves were also collected from Exp. 5. The fourth and fifth leaf from the top were collected after five weeks, two hours after lights went on. Two plants were pooled and four pools per genotype were made. Gene expression was determined using qPCR (quantitative reverse transcription polymerase chain reaction). Frozen leaf material was ground and used for RNA extraction with an RNeasy plant mini-kit (Qiagen). A spectrophotometer (NanoDrop, Thermoscientific, Thermofisher) determined RNA concentration and quality. A DNase treatment was performed using Amplification grade DNase I (Invitrogen, Thermofisher). 1µg of RNA was used for cDNA synthesis with an iScript kit (Bio-rad). RNA extraction, DNase treatment and cDNA synthesis were performed as described in the supplied manufacturer's protocols. 20µl of cDNA was diluted to a total volume of 150 µl. 5µl of SYBR-green (iQ-SYBR-green super mix, Bio-Rad), 0.25µl Forward Primer (10µM), 0.25µl Reverse Primer (10µM), 0.5µl Milli-Q water and 4µl diluted cDNA were used for the qPCR. In Exp. 3 three technical replicates were used per pooled sample. Samples were placed in a Thermal Cycler (C1000, Bio-Rad) set to 95°C for 3 minutes,

40 cycles of 95°C for 15 seconds and 60°C for 1 minute, followed by 95°C for 10 seconds and for a melt curve 65°C to 95°C in 0.5°C steps every 5 seconds. Primers used were: *StSP6A* (PGSC0003DMT400060057): (F) GACGATCTTCGCAACTTTTACA, (R) CCTCAAGTTAGGGTCGCTTG and *StSP3D* (scaffold PGSC0003DMB00000014, unannotated): (F) GGACCCAGATGCTCCAAGTC, (R) CTTGCCAAAACTTGAACCTG and *StNAC* (reference gene *NASCENT POLYPEPTIDE-ASSOCIATED COMPLEX ALPHA*, PGSC0003DMT400072220): (F) ATATAGAGCTGGTGATGACT, (R) TCCATGATAGCAGAGACTA. Primers for *StSP6A* and *StSP3D* were used in (Navarro et al., 2011) and the *StNAC* primer had an efficiency of 99%.

Data analysis

A student's t-test was used to compare two treatments and a one-way analysis of variance (ANOVA) was used to compare more than two treatments. A Bonferonni pair-wise comparison was used to determine which treatments significantly differed ($\alpha = 0.05$, IBM, SPSS Statistics 22 and GenStat, 18th Edition). When data was ordinal or not normally distributed (tested with a Shapiro-Wilk W-test for non-normality in GenStat), a non-parametric Kruskal-Wallis test and Dunn's pairwise comparisons ($\alpha = 0.05$) were computed in SPSS. Comparisons between light treatments in Exp. 2 and 3 were based on biological replicates as independent experimental units. For gene expression analysis three technical replicates were used for the qPCR analysis in Exp. 4 and four biological replicates were used for qPCR analysis in Exp. 5. $100/2^{-\Delta Ct}$ was used to determine gene expression values. Ct (cycle threshold) values of the gene of interest (*StSP6A* and *StSP3D*) were used to determine expression of the gene of interest compared to the housekeeping gene *StNAC*. Invariant expression of *StNAC* under the tested conditions is shown in Supplementary Fig. S1.

RESULTS

Removing the tuber-sink: grafting onto a non-tuberizing rootstock

In order to establish how the absence or presence of tubers would affect flowering of the scions, two grafting experiments were performed in short-day and long-day conditions.

Grafting under short day conditions

306 Short-day conditions strongly promote tuberization. To determine whether flower bud
307 development in CE3027 would improve without tubers, we grafted CE3027 scions onto non-
308 tuberizing *S. etuberosum* and *S. palustre* rootstocks and grew them in short-day conditions.
309 CE3027 scions underwent floral transition and as expected the flower buds failed to develop
310 in the control grafts with tuberizing CE3027 rootstocks. Moreover, the buds also failed to
311 develop when the CE3027 scion was grafted onto the non-tuberizing *S. etuberosum* or *S.*
312 *palustre* rootstocks. Thus, the absence of tubers could not improve flower bud development.
313 Opposite grafts were made with *S. etuberosum* and *S. palustre* scions on CE3027 rootstocks to
314 determine how tubers would affect flower bud development. However, in the short-day
315 conditions, neither *S. etuberosum* nor *S. palustre* transitioned to flowering and the CE3027
316 rootstocks failed to tuberize. To gain a better understanding on the effect of tubers on flower
317 bud development, and attempt to induce flowering in *S. etuberosum,* a grafting experiment was
318 performed under long-day conditions.
319
320 *Grafting under long day conditions*
321 Grafting CE3027 scions onto non-tuberizing *S. etuberosum* rootstocks did not improve flower
322 bud development compared to the control grafts, which tuberized (CE3027 scion on CE3027
323 rootstock) (Fig. 2). Surprisingly, the opposite effect was observed. Although all tested graft
324 combinations underwent flower transition (data not shown), grafts with CE3027 scions on *S.*
325 *etuberosum* rootstocks only reached anthesis in four of the eight (50%) plants, whereas nine of
326 the eleven (>80%) control grafts reached anthesis (Fig. 3). Furthermore, the grafts with *S.*
327 *etuberosum* rootstocks had almost half the number of open flowers compared to control grafts.
328 The grafts made with scions of the early tuberizing genotype CE3130 also had impaired flower
329 bud development in grafts with non-tuberizing rootstocks (Supplementary Table S2A).
330 To determine if the presence of tubers would impair *S. etuberosum* flower bud development,
331 we made opposite grafts with *S. etuberosum* scions on tuberizing CE3027 rootstocks. In
332 contrast to the short-day grafting experiment, flower transition occurred in *S. etuberosum* and
333 the flower buds developed into open flowers. Furthermore, CE3027 rootstocks tuberized, even
334 when *S. etuberosum* scions were grafted onto them. However, a larger fraction of grafts with
335 *S. etuberosum* scions on tuberizing CE3027 rootstocks reached anthesis, than of control grafts
336 with *S. etuberosum* scions on non-tuberizing *S. etuberosum* rootstocks (Table 1). When
337 comparing grafts in which the CE3027 rootstock was completely defoliated, with grafts in
338 which some leaves were kept below the graft junction, the presence of leaves accelerated
339 tuberization in CE3027 rootstocks with approximately nine days (data not shown). Also, a

larger fraction of grafts with leafy CE3027 rootstocks reached anthesis than grafts with leafless CE3027 rootstocks. Grafts with *S. etuberosum* scions on the early tuberizing CE3130 rootstocks showed a similar result. Anthesis was higher in grafts with CE3130 rootstocks and the presence of leaves accelerated tuberization, and also increased the number of plants with open flowers. (SupplementaryTable S2B). Thus, tuberizing rootstocks did not impair the flower bud development of *S. etuberosum* scions.

Taken together, the interspecific grafting experiments did not show that the presence of tuber sinks impaired flower bud development, but rather had an unexpected opposite outcome where an improved flower bud development was observed in grafts producing tubers. To validate that these results were not due to interspecific interaction in the grafts, we performed another experiment where the tuber-sink was removed within the same genotype.

Removing the tuber-sink: removing stolons

To determine whether tubers negatively influenced flower bud development, tuberization was prevented by removing the stolons in CE3027 plants. Removing stolons did not significantly affect the number of flowering plants nor the number of open flowers per plant (Table 2). Also, the time until anthesis was not affected by removing the stolons (data not shown). The light conditions under which plants were grown did affect flower bud development. The number of flowering plants was low in low-light short days (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$), but in high-light short days (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) the number of flowering plants was almost as high as in long days (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Nevertheless, removing stolons did not improve flower bud development, both under short-day and long-day conditions.

These results confirm that short-day conditions impair flower bud development in CE3027. Although the number of flowering plants was similar in a high-light short day compared to a low-light long day (SD400 and LD200 had the same daily light sum), the maximum number of open flowers per plant was significantly higher in the long day treatment (1.2 flowers in SD400 vs. 6.5 in LD200).

Tuberization took place in all light treatments unless stolons were removed (Table 2). The short-day treatment with high light intensity resulted in the fastest tuberization and the highest tuber biomass. Plants without stolons had a higher shoot biomass than plants with stolons. The light treatments with the highest light sum (SD400 and LD200) had a higher shoot biomass than the low light sum short day (SD200), in both tuberizing and non-tuberizing plants. The total biomass (tuber + shoot) was highest in the high-light short-day treatment.

In summary, preventing tuberization by removing the stolons did not improve flower bud development, even though flower bud development was impaired in short days.

Removing the tuberization signal: reducing *StSP6A* expression

To determine whether the tuberization signal negatively influenced flower bud development, we used transgenic plants with reduced expression of the tuberization signal *StSP6A*. Flower buds were formed in all *S. andigena* plants, but flower bud development of the *S. andigena* wild type was impaired in short days compared to long days (Fig. 4). Wild-type plants under high-light short-day and low-light long-day conditions (SD400 and LD200) received the same daily light sum, but flower buds were smaller in the short-day treatment (Fig. 5A, $P = 0.02$). In the *StSP6A RNAi* lines grown under short-day conditions, flower bud development was improved compared to the wild type. Under these conditions, flower bud development in the *StSP6A RNAi* lines equaled the level of flower bud development in the wild-type plants under long-day conditions (Fig 5A, no significant difference between short day *StSP6A RNAi* lines and long day wild-type lines, $P = 0.12$). Moreover, two of the five *StSP6A RNAi* #13 plants in the high-light short days reached anthesis, which did not occur in *S. andigena* wild-type or *StSP6A RNAi* plants in any other treatment, not even in long days (Fig. 5B). In long days, a lower *StSP6A* expression did not have an effect on flower bud development. Reducing *StSP6A* expression did not affect the flower transition time in either short or long days. Gene expression analysis of *StSP6A* in *StSP6A RNAi* lines show that these lines were indeed silenced in *StSP6A* (Supplementary Fig. S2A). As expected, tuberization in the transgenic lines with reduced *StSP6A* expression, was inhibited compared to the wild-type plants (Supplementary Fig. S2B). Wild-type plants in high-light long-day conditions showed a later and reduced tuberization compared to the plants in short days, while low-light long-day plants did not tuberize at all. In summary, our results show that inhibiting tuberization by reducing *StSP6A* expression in potato plants grown under short-day conditions improves flower bud development.

Removing the tuberization signal and the tuber-sink: reducing *StSP6A* expression and removing stolons

As the experiments testing the removal of the tuber-sink used different genotypes than the experiments testing removal of the tuberization signal *StSP6A*, we performed a short-day experiment with *S. andigena*, where the tuberization signal *StSP6A* and the stolons were removed. Also, flower bud development was measured in more detail, to better illustrate differences between treatments. Plants with reduced levels of *StSP6A*, clearly developed larger

flower buds than wild-type plants, as in Exp. 3 (Fig. 6). The only plant to reach anthesis was a *StSP6A RNAi* plant without stolons. However, only removing the stolons did not significantly affect the flower bud size. Reducing *StSP6A* expression or removing the stolons did not affect the flower bud appearance time, which occurred on average after 28 days in all treatments (data not shown). The results show flower bud development is improved when the tuberization signal is removed, but not when only tubers are removed.

Increasing the tuberization signal: overexpressing *StCDF1* in long days

StCDF1 overexpressing lines in a CE3027 background were used to confirm that *StSP6A* impairs flower bud development. Both *StCDF1* overexpressing lines in long days had upregulated *StSP6A* expression compared to the wild type (Fig. 7A). The flower bud development in these lines was inhibited and no plants reached anthesis (zero of the 16 plants) (Fig. 7B). The wild-type CE3027 plants were able to reach anthesis in long days (five of the seven plants had open flowers, one plant died).

All experiments: plant growth after removing the tuber-sink

In the experiments where tubers were removed, but the plants remained induced to tuberize, the plants showed abnormal growing patterns. In the grafting experiments in short days, scions of tuberizing genotypes on non-tuberizing rootstocks formed aberrant side-shoots. Although these structures were green and lacked the characteristic hook found on stolon tips, they resembled stolons (Fig. 8A-B). These "aerial stolons" grew towards the soil and in some cases, once reaching the soil, formed tubers at the tip (Fig. 8B). These stolon-like structures were also found in long days, in grafts with scions of the early-tuberizing CE3130 on non-tuberizing rootstocks. Stolon-like structures also formed on stems of potato plants in inducing short days (Fig. 8C-D), where stolons were removed. In some cases, tubers formed directly on the stem (Fig. 8D). Potato plants that were induced to tuberize, but unable to do so in the conventional way, found alternative means of tuberization.

DISCUSSION

Grafting with non-tuberizing rootstocks did not improve flower bud development

Long-day grafts with *S. etuberosum* rootstocks did not form tubers, but reached anthesis less often than the tuberizing control grafts and produced less open flowers when anthesis was

reached (Fig. 3A-B). This is in line with results in opposite grafts, where the effect of tuberizing rootstocks on *S. etuberosum* scions was tested; in these grafts the fraction of plants with open flowers increased compared to control grafts with *S. etuberosum* rootstocks (Table 1). The results show that removing the tuber-sink does not improve flower bud development.

That tuberizing rootstocks did not impair, but improved flower development in *S. etuberosum* scions, was surprising. Instead of inhibiting flower development, tuberization may improve flowering in a different species (*S. etuberosum*). The FT of one species can induce flowering or tuberization in another species, for instance with rice *Heading date 3a* (*Hd3a)* in potato, *Arabidopsis FT* in tomato and tobacco, and tomato *SINGLE FLOWER TRUSS (SFT)* in Arabidopsis (Lifschitz *et al*., 2006; Lifschitz and Eshed, 2006; Navarro *et al*., 2011). Potato StSP6A from the rootstock may improve *S. etuberosum* flowering in the scion. Interestingly, long-day grafts between *S. etuberosum* scions and leafless CE3027 or CE3130 rootstocks flowered and tuberized, while short-day grafts between *S. etuberosum* scions and the leafless CE3027 and CE3130 rootstocks did not (data not shown). Perhaps in long days, a leaf-derived FT from *S. etuberosum* induces tuberization, while in short days this signal is not produced. Potato plants are thought to have an auto regulatory *StSP6A* loop, where leaf-derived StSP6A leads to upregulation of *StSP6A* in the stolons, enhancing the level of StSP6A for tuberization (Navarro *et al*., 2011). Potato scions expressing rice *Hd3a* but no *StSP6A* have induced *StSP6A* in the stolons (Navarro *et al*., 2011). If FT from *S. etuberosum* also induces this auto regulatory loop, *S. etuberosum* FT may induce tuberization in the CE rootstocks and amplify the amount of FTs in the graft, possibly enhancing flowering as well.

Most importantly our grafting experiments show that the tuber-sink does not impair flower bud development. However, because interspecific grafts were used, effects on flowering may have been caused by other properties of the *S. etuberosum* than its inability to tuberize. Therefore, to determine whether removing the tuber-sink improves flower bud development, stolons were removed in potato plants.

Removing stolons did not improve flower bud development

As with grafting, removing the stolons did not improve flower bud development in both CE3027 and *S. andigena* genotypes (Table 2 and Fig. 6). This is in line with previous experiments on stolon abscission (Weinheimer and Woodbury, 1966). Removing stolons also had no effect on flower initiation. The lack of stolons did lead to an increase of assimilates available for the shoot, as seen in the significant increase in shoot biomass (Table 2). However, this increase in shoot biomass did not improve flower bud development.

In short days, flower bud development was impaired compared to long days (Table 2). However, by raising short-day light intensity to match the light sum of long days, the fraction of plants to reach anthesis increased from two out of eleven plants (< 20%) to ten out of eleven plants (> 90%), which almost rivalled long-day anthesis (anthesis in all plants). Sufficient light is crucial for flower bud development, as has been demonstrated in several crops including potato and tomato (Kinet, 1977; Demagante and Zaag, 1988; Turner and Ewing, 1988). Increasing light may increase the amount of assimilates formed in the plant. Assimilates like sucrose play an important role in flower induction and floral development in potato (Chincinska *et al.*, 2008). Nevertheless, the number of open flowers was significantly higher under long-day conditions, indicating an impairment of CE3027 flowering in short days, as was found before in other potato genotypes (Turner and Ewing, 1988). Thus, short-day flower bud development was impaired and preventing formation of the tuber-sink by removal of the stolons did not improve this development.

Removing the tuber-sink in a plant that was induced to tuberize led to "aerial stolons"

Removing the tubers did not improve flower bud development. Although the plants were unable to tuberize, they were still induced to do so. Grafts that could not tuberize, started to produce stolon and tuber-like structures on the scions (Fig. 8A-B). Plants without stolons, growing in short days, also made stolon-like structures on the stem (Fig. 8C-D). Alternative tuberization structures have been documented before (Thijn, 1954; Weinheimer and Woodbury, 1966) in conditions where tuberization is prevented but plants remain induced to tuberize. The lack of tubers led to more assimilates in the shoot, but instead of promoting flowering these assimilates may have been directed towards alternative tuberization structures. The tuberization signal *StSP6A* is still expressed in inducing conditions, even when tubers are removed, which may be the cause of the direction of assimilates to alternative tuberization structures instead of to the flower buds. This theory is supported by the finding that the formation of stolon-like structures in short-day *StSP6A RNAi* plants was much less severe.

The tuberization signal StSP6A impairs flower bud development

S. andigena wild-type plants underwent floral transition in all tested light treatments, but in short days the flower buds ceased to develop at a very early stage (Fig. 5A). Remarkably, short-day flower bud development was significantly improved in the *StSP6A RNAi* lines. Two of the *StSP6A RNAi* plants were even able to reach anthesis in short days, which did not happen in any other treatment and is uncommon for *S. andigena* when grown in our climate chamber

conditions. Flowering in *StSP6A RNAi* plants was also tested by Navarro *et al.* (2011), but only transition to flowering was considered and not flower bud development. The transition to flowering occurred at the same time as in the wild type, as was the case in our experiments (data not shown). The transgenic lines had a significantly reduced *StSP6A* expression (Supplementary Fig. S2A). Improved flower bud development in the transgenic lines could not be explained by an increase in transcription of the proposed flowering signal *StSP3D* in the leaves (data not shown), implying that *StSP6A* negatively affects flower bud development through a different mechanism.

StSP3D has been proposed to be the flowering signal in potato, because silencing *StSP3D* showed a late flowering response (Navarro *et al*., 2011). However, there is a lack of correlation between flower bud development and *StSP3D* expression, which is strongly expressed under short day conditions but weakly expressed under long day conditions (in prep. Dr. S. Bergonzi). Perhaps low expression levels of *StSP3D* are sufficient to induce flowering and the level of *StSP6A* determines the success of flower bud development. To fully understand potato flowering, elucidating the role of StSP6A in flower bud development, as well as StSP3D in flowering time and development, will be crucial.

Our finding that StSP6A represses flower bud development, while the tuber-sink does not, was confirmed in another experiment testing both stolon abscission (tuber-sink) and silencing of *StSP6A* (tuberization signal) in *S. andigena* in short days. Removal of stolons did not improve flower bud development, while downregulation of *StSP6A* did. The repressing role of StSP6A on flower bud development was further confirmed in CE3027 *StCDF1* overexpressing lines, with upregulated *StSP6A* in long days. The flower bud development was impaired in these lines and resembled the impaired flower bud development found in wild-type *S. andigena* plants in short days. Transgenic lines in which an upstream regulator of *StSP6A* was overexpressed were used instead of *StSP6A* overexpressing lines, to induce *StSP6A* in long days. In Navarro (2011), transgenic lines overexpressing *StSP6A* actually improved flowering, perhaps by the strong and ubiquitous expression of *StSP6A* by the 35S promotor (Odell *et al.,* 1985; Seternes *et al.*, 2016). In the *StCDF1* overexpressing lines, the down-stream regulation on *StSP6A* is still intact, allowing a more realistic upregulation of *StSP6A* than in a *35S::StSP6A* overexpressing line. Flower impairment in these lines confirms our earlier findings that StSP6A represses flower bud development.

Can the inhibiting effect of a tuber-sink be ruled out?

Although flower bud development was not improved by tuber-sink removal in CE3027, CE3130 or *S. andigena*, removing the tuber-sink had a positive effect on flowering in some genotypes in the past (Thijn, 1954; Jessup, 1958). However, these reports have also been contradicted (Turner and Ewing, 1988). Therefore, it may be possible that repression of flower development by the tuber-sink is genotype specific. It would be interesting to find out if reducing *StSP6A* would further improve flower development in genotypes that are benefitted by tuber-sink removal. Nevertheless, our findings show that in *S. andigena* and CE3027 the tuber-sink does not repress flower bud development while the tuberization signal StSP6A does.

The day-length control of flowering in potato

Short days, or more correctly long nights, induce tuberization in potatoes, although variation exists between varieties in their dependence on short days (Garner and Allard, 1923; Ewing and Struik, 1992; Prat, 2010; Kloosterman *et al.*, 2013). Potato flowering has been categorized as a short-day, long-day and day-neutral process (Jones and Borthwick, 1938; Turner and Ewing, 1988; Almekinders and Struik, 1994; Martínez-García *et al.*, 2002; Schittenhelm *et al.*, 2004). A cause for this variation might be the use of different genotypes and the difference in defining flowering. Because flowering is a process composed of many phases, it needs a clear distinction when addressed: it starts with flower transition and proceeds with flower bud and organ development. More importantly, the flowering process is not only influenced by day length but by tuberization as well, which varies between genotypes. Our results show that the floral transition occurs independently of the photoperiod but that flower bud development is repressed by the tuberization signal. Remarkably anthesis was only attained in short days with high irradiance (in *StSP6A RNAi*#13) indicating that environmental growing conditions also play a role in the process. Our results point to a short-day control of flower bud development in potato, but due to internal control by StSP6A, flower bud development is promoted under long-day conditions.

Interaction between two modes of reproduction in potato

A likely mode of action for StSP6A to impair flower bud development, could be through control of assimilates. Although removing the tuber-sink did not improve flower bud development, it cannot be claimed assimilates do not play a role, as alternative tuber structures that acted as sinks were still formed unless *StSP6A* was silenced. StSP6A may have a role in directing assimilates towards tuberization, which consequentially could be detrimental for flower development, especially if tuberization takes place while flower buds are still

developing. How this direction of assimilates takes place remains to be elucidated. Whether flowering is actually repressed by StSP6A may be genotype specific and depend on the timing of both tuberization and flowering. The European Cultivated Potato Database (https://www.europotato.org) shows a huge variation in flowering success between varieties and it has been suggested that potato berry and seed development is impeded by earliness of tuberization (Pallais, 1987). Similar findings were seen in the CE3027 and CE3130 control grafts, where the early tuberizing CE3130 grafts flowered less profusely than the later tuberizing CE3027 grafts (Fig. 3B and Supplementary Table S2). It would be interesting to correlate the tuberization time and *StSP6A* expression to the flowering time and flower developmental success in a large number of genotypes.

While two reproduction modes may inhibit each other in the same species, interspecies interaction between reproduction modes may be beneficial for both processes, as was seen in *S. etuberosum* scions grafted on CE3027 and CE3130 rootstocks. The flowering in *S. etuberosum* scions was improved compared to control grafts with *S. etuberosum* rootstocks. StSP6A may not function as an inhibiter in *S. etuberosum* because flowering and tuberization are not competing processes in this species. Consequently, StSP6A may substitute FT in *S. etuberosum* and improve flowering, while StSP6A inhibits flowering in potato.

Conclusion

Our results show that flower bud development in potato is impaired by the tuberization signal StSP6A, and not by the tuber-sink itself. These results suggest there is an internal mechanism in potato plants where one mode of reproduction can affect the other.

SUPPLEMENTARY DATA

Table S1. Overview of the five experiments testing how tuberization affects potato flower bud development.

Table S2. Flower bud development and tuberization in grafts between CE3130 and *S. etuberosum*.

Figure S1. *StNAC* expression (Ct = cycle threshold) in wild-type *S. andigena, StSP6A RNAi* #1, and *StSP6A RNAi* #13.

Figure S2. *StSP6A* expression and tuberization time in wild-type *S. andigena, StSP6A RNAi*

#1, and *StSP6A RNAi* #13.

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Table 1. Flower bud development and tuberization in grafts with *S. etuberosum* scions and *S. etuberosum* or CE3027 rootstocks (with or without leaves) (Exp. 1).

	Rootstock	*n*	Anthesis *	Max. open flowers/plant	Tuber dry weight (g/plant)
Scion *S. etuberosum*	*S. etuberosum* (non-tuberizing)	3	1	3.3 ±3.1 a**	0.00 ±0.00 a
	CE3027 (tuberizing)	9	3	3.0 ±3.3 a	1.03 ±1.95 a
	CE3027 + leaves (tuberizing)	10	5	1.7 ±3.1 a	1.77 ±1.96 a

* Plants that reached the open flower stage

** Standard deviations are given, identical alphabetical letters indicate no significant difference between graft combinations ($\alpha = 0.05$)

Table 2. The effect of removing stolons on CE3027 flowering and plant biomass in different light treatments. Biological replicates, *n* = 11. (Exp. 2).

Light treatment	Stolons	Anthesis *	Max. open flowers/plant	Tuber dry weight (g/plant)	Shoot dry weight (g/plant)	Shoot + tuber dry weight (g/plant)
SD200**	Intact	2	0.3 ±0.6 a***	4.2 ±0.9 b	2.2 ±0.2 a	6.5 ±1.0 b
	Removed	2	0.2 ±0.4 a	0.0 ±0.0 a	5.5 ±0.9 c	5.5 ±0.9 a
SD400	Intact	10	1.2 ±1.0 ab	10.1 ±1.1 c	2.4 ±0.2 ab	12.5 ±1.2 c
	Removed	10	2.4 ±1.0 b	0.0 ±0.0 a	7.8 ±1.8 d	7.8 ±1.8 b
LD200	Intact	11	6.5 ±1.5 c	4.1 ±1.4 b	3.5 ±0.3 b	7.6 ±1.5 b
	Removed	11	6.2 ±1.0 c	0.0 ±0.0 a	7.8 ±1.1 d	7.8 ±1.1b

* The number of plants that reached the open flower stage

** Number indicates light intensity in $\mu mol \cdot m^{-2} \cdot s^{-1}$, SD = short day (8 hours), LD = long day (16 hours)

*** Standard deviations are given, identical alphabetical letters indicate no significant difference between treatments ($\alpha = 0.05$)

Figure 1. The three methods used to eliminate tuberization in potato and determine whether flower bud development is improved. (1) Potato plant scions that are able to tuberize are grafted onto non-tuberizing wild potato rootstocks (2) The stolons of the potato plant are removed as soon as they appear. (3) The tuberization signal StSP6A, which is expressed in the leaves, is silenced in transgenic lines.

Figure 2. Schematic representation of flowering and tuberization in the grafting combinations between a tuberizing and non-tuberizing genotype, in long days. Potato genotype CE3027 is able to tuberize, while *S. etuberosum* is unable to tuberize. (A) The control grafts of CE3027 made tubers. (B) Grafts with CE3027 scions and *S. etuberosum* rootstocks did not make tubers. (C) The control graft of *S. etuberosum* did not make tubers. (D) Grafts with *S. etuberosum* scions and CE3027 rootstocks did make tubers, with or without leaves on the rootstock. All graft combinations formed buds which developed into open flowers. The graft combinations with a tuberizing rootstock (A, D) formed more open flowers than grafts without tuberizing rootstocks (B, C). (Exp. 1).

Figure 3. Flower bud development and tuber biomass in grafts with potato scions (CE3027) and tuberizing rootstocks (CE3027) or non-tuberizing rootstocks (*S. etuberosum*) in long days. (A) The percentage of grafts that reached anthesis (open flowering stage), absolute numbers are indicated in the bar. (B) The maximum number of open flowers on a plant. (C) The dry weight of the tubers per plant at harvest, NT = no tuberization (biomass 0). The asterisk represents a significant difference between grafts with a tuberizing rootstock and a non-tuberizing rootstock, $\alpha = 0.05$. Error bars show standard deviations. (Exp. 1). $n = 11$ (CE3027/CE3027) and $n = 8$ (CE3027/*S. etuberosum*).

Figure 4. Flower buds in *S. andigena* in short and long days. Flower buds in high-light short days (SD400, 8/16 hours light/dark, 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and low-light long days (LD200, 16/8 hours light/dark, 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) five weeks after transplanting and eight weeks after transplanting. (Exp. 3).

Figure 5. The effect of photoperiod and light intensity on flower bud development in *S. andigena* wild-type and *StSP6A RNAi* plants. Four light treatments were used: SD200 (short day, 8/16 hours light/dark, 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$), SD400 (short day, 8/16 hours light/dark, 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$), LD200 (long day, 16/8 hours light/dark, 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and LD400 (long day, 16/8 hours light/dark, 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$). SD400 and LD200 have the same daily light sum. (A) Flower bud development was categorized by size where 0 was no bud and 5 was an open flower. The median of the furthest stage of bud development during growing is given. Identical letters

indicate no significant difference between genotypes in a light treatment (α = 0.05). Biological replicates *S. andigena, n* = 8 and *StSP6A RNAi* lines, *n* = 5. (B) Plants at harvest. A wild-type *S. andigena* in SD400, a *StSP6A RNAi* #13 plant in SD400, a wild-type *S. andigena* in LD200 and a *StSP6A RNAi* #13 plant in LD200. (Exp. 3).

Figure 6. The flower bud development in *S. andigena* in wild-type and *StSP6A RNAi*#13 lines where the stolons were either left intact or removed. Plants were grown in short days (8/16 hours light/dark) with a light intensity of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. (A) Flower bud size during growth (measurements were ceased when flower bud abortion started) and (B) maximum flower bud size reached by the plant. Error bars show standard deviations. Letters indicate significant differences in maximum flower bud size between treatments (α = 0.05). Biological replicates, *n* = 10. WT = wild type. (C) Flower buds six weeks after transplanting, in WT *S. andigena* and *StSP6A RNAi*#13 *S. andigena*, with stolons intact or removed. (Exp. 4).

Figure 7. *StSP6A* expression and flowering phenotypes in wild-type CE3027, *35S::StCDF1* #3 and *35S::StCDF1* #4. Plants were grown in long days (16/8 hours light/dark) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (A) *StSP6A* is expressed relative to the reference gene *StNAC*. Expression is from plants five weeks after transplanting. The error bars show the standard deviations. Alphabetical letters indicate significant differences between genotypes (α = 0.05). Biological replicates, *n* = 4. (B) The shoot apex in wild-type and transgenic CE3027 plants eight weeks after transplanting. Genotypes from left to right: wild type*, 35S::StCDF1* #3 and *35S::StCDF1* #4. The number of plants that reached anthesis after eight weeks is indicated. (Exp. 5).

Figure 8. Stolon-like side-shoots formed under tuber inducing conditions if tuberization was impaired. (A) A graft where the scion of a plant that was able to tuberize was grafted onto a wild non-tuberizing rootstock (CE3027 / *S. palustre*) in short days. Stolon-like structures are formed above the graft unison (white arrow). (B) In the same graft combination the stolon-like structures on the scion formed a tuber upon reaching the soil. (C) In the CE3027 plants where the stolons were removed, stolon-like structures were formed aboveground on the stem. (D) *S. andigena* plants grown in short days where the stolons were removed, formed tuber-like structures directly on the stem.

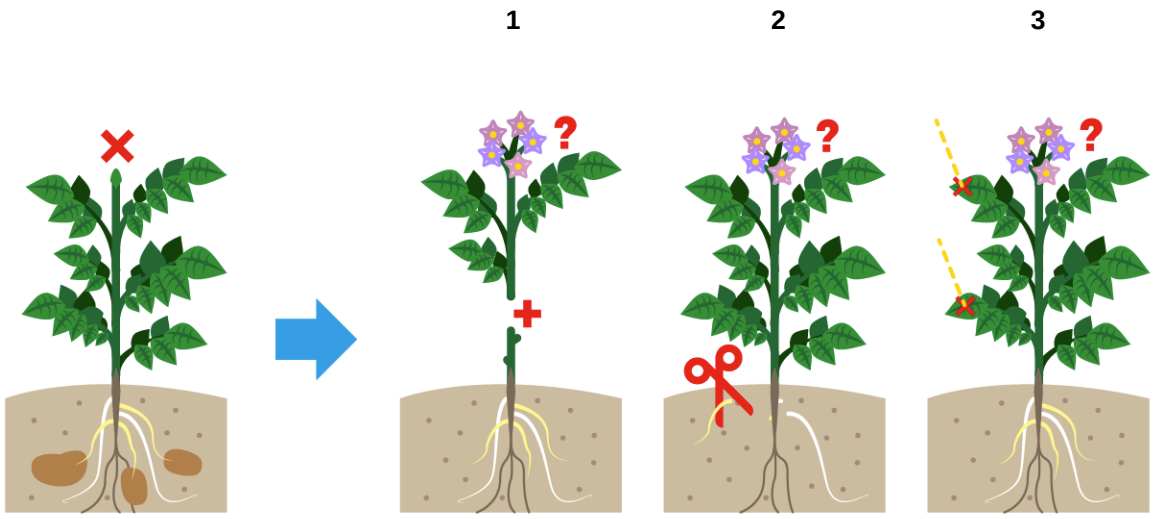

Figure 1.

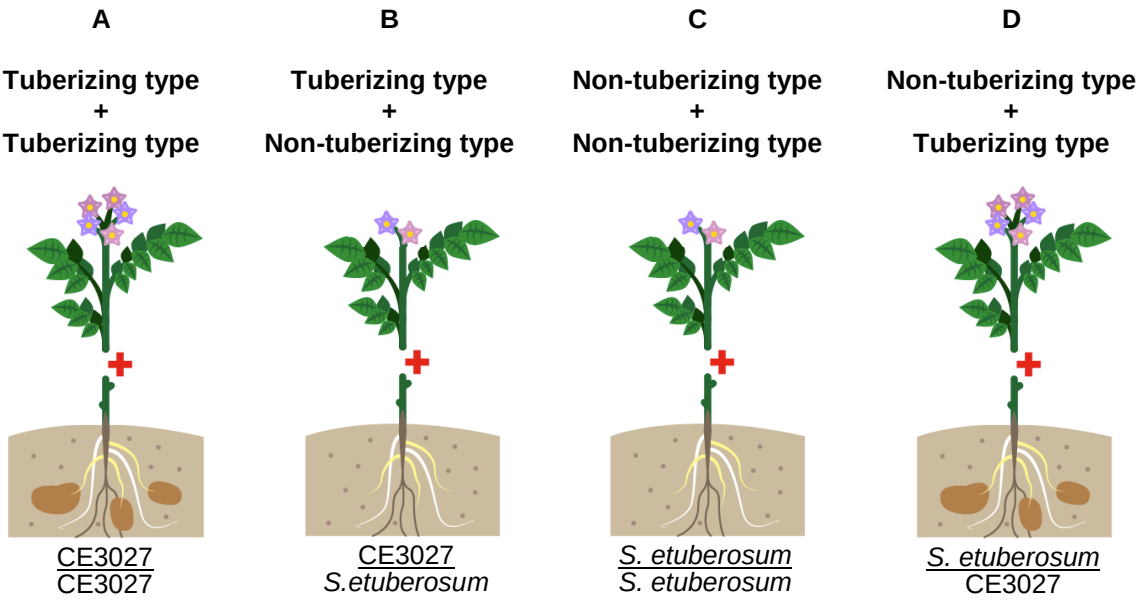
A
Tuberizing type
+
Tuberizing type
CE3027
CE3027
B
Tuberizing type
+
Non-tuberizing type
CE3027
S.etuberosum
C
Non-tuberizing type
+
Non-tuberizing type
S. etuberosum
S. etuberosum
D
Non-tuberizing type
+
Tuberizing type
S. etuberosum
CE3027

Figure 2.

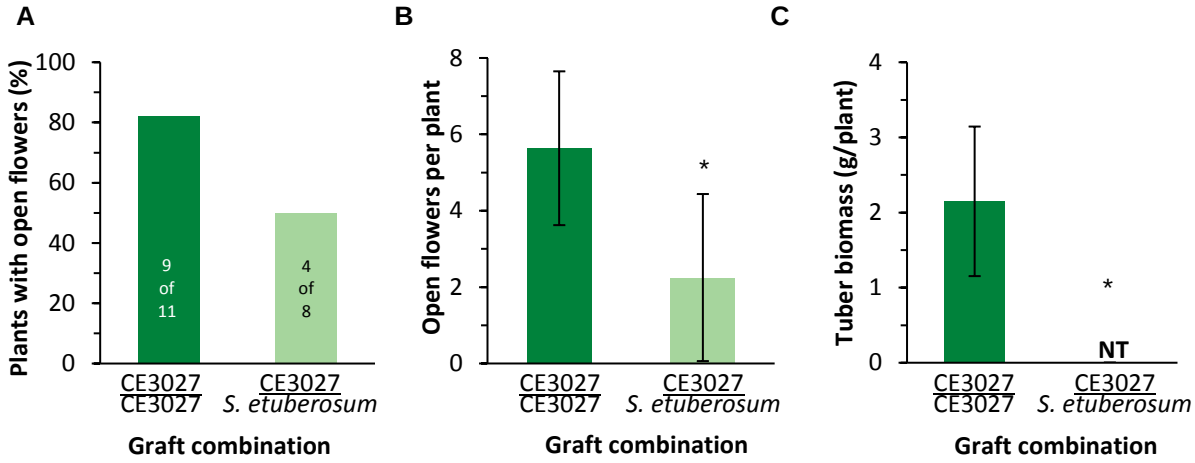
A
B
C
Plants with open flowers (%)
100
80
60
40
20
0
9
of
11
4
of
8
CE3027
CE3027
CE3027
S. etuberosum
Graft combination
Open flowers per plant
8
6
4
2
0
*
CE3027
CE3027
CE3027
S. etuberosum
Graft combination
Tuber biomass (g/plant)
4
3
2
1
0
*
NT
CE3027
CE3027
CE3027
S. etuberosum
Graft combination

Figure 3.


WT S. andigena 5wk
SD400
WT S. andigena 5wk
LD200
WT S. andigena 8wk
SD400
WT S. andigena 8wk
LD200

Figure 4.

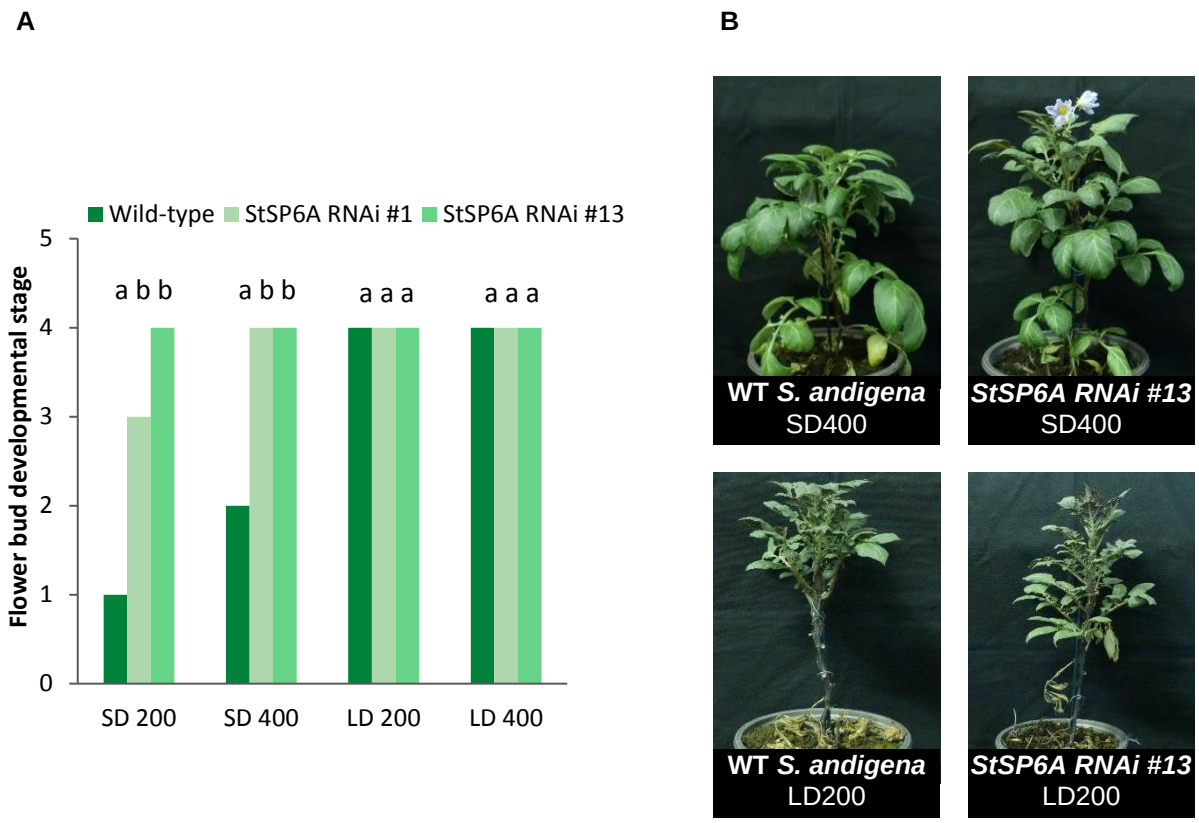
A
B
Wild-type
StSP6A RNAi #1
StSP6A RNAi #13
Flower bud developmental stage
0
1
2
3
4
5
a b b
a b b
a a a
a a a
SD 200
SD 400
LD 200
LD 400
WT S. andigena
SD400
StSP6A RNAi #13
SD400
WT S. andigena
LD200
StSP6A RNAi #13
LD200

Figure 5.

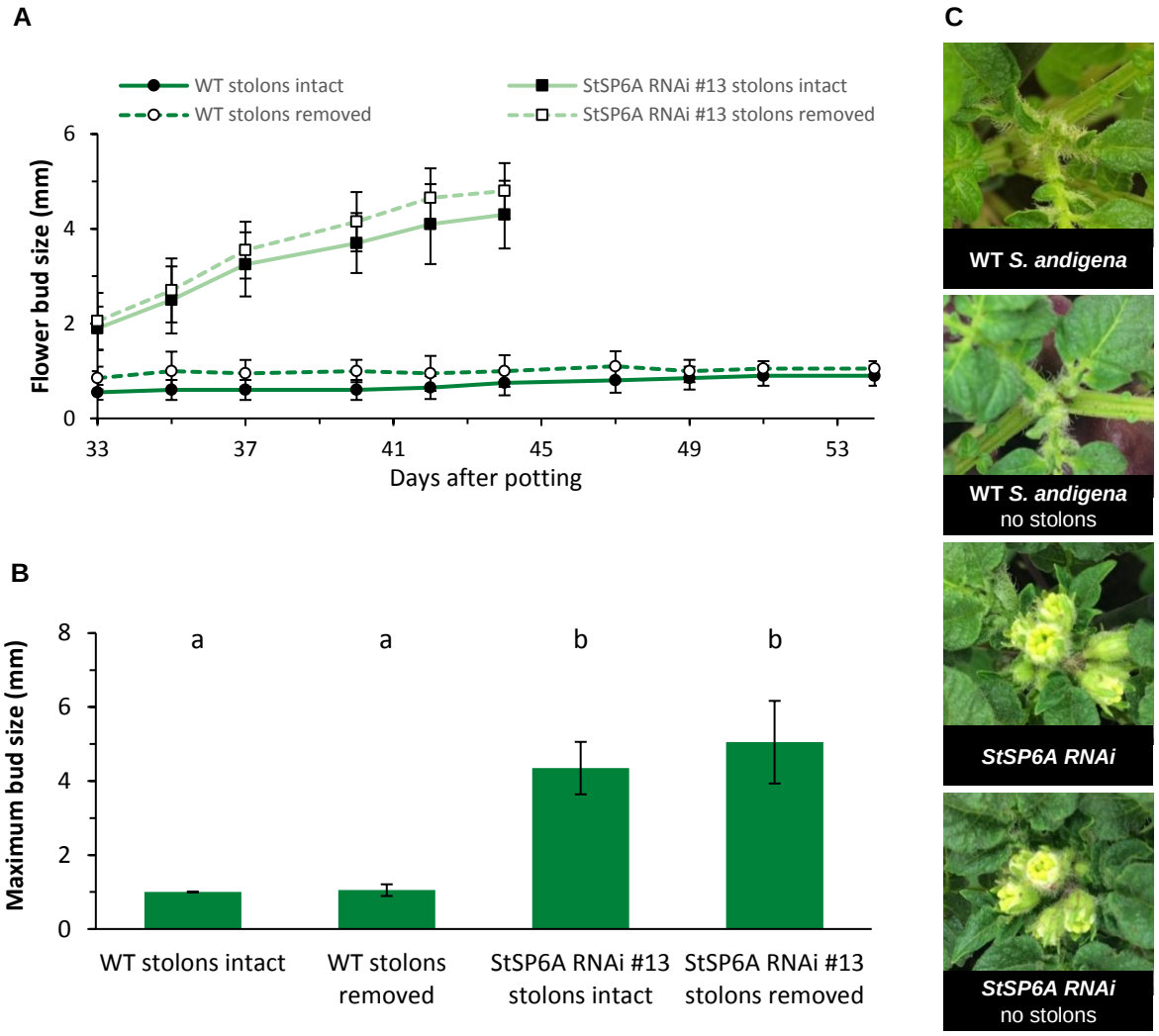
A
WT stolons intact
WT stolons removed
StSP6A RNAi #13 stolons intact
StSP6A RNAi #13 stolons removed
Flower bud size (mm)
0
2
4
6
33
37
41
45
49
53
Days after potting
B
Maximum bud size (mm)
0
2
4
6
8
a
a
b
b
WT stolons intact
WT stolons removed
StSP6A RNAi #13 stolons intact
StSP6A RNAi #13 stolons removed
C
WT S. andigena
WT S. andigena no stolons
StSP6A RNAi
StSP6A RNAi no stolons

Figure 6.

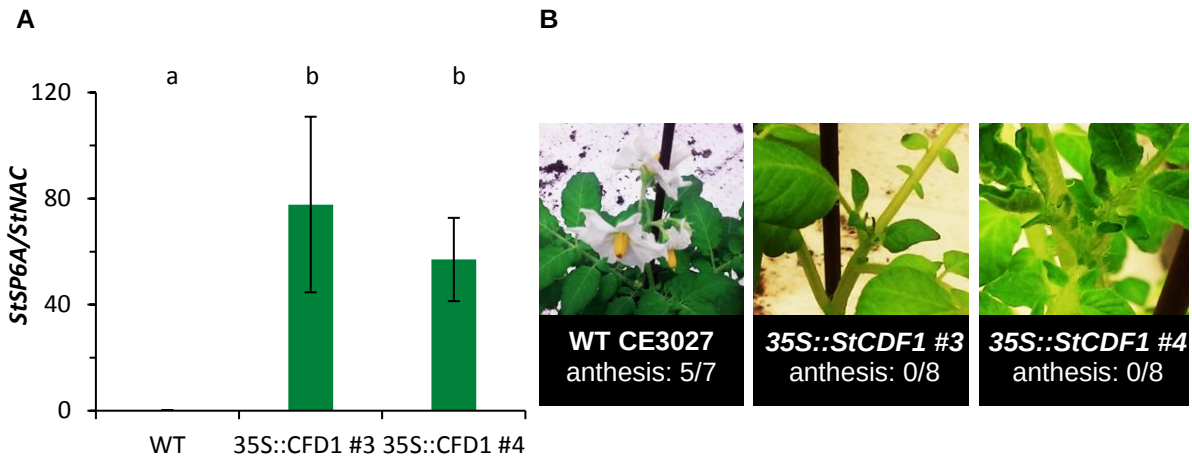
A
B
a
b
b
120
80
40
0
StSP6A/StNAC
WT
35S::CFD1 #3
35S::CFD1 #4
WT CE3027
anthesis: 5/7
35S::StCDF1 #3
anthesis: 0/8
35S::StCDF1 #4
anthesis: 0/8

Figure 7.

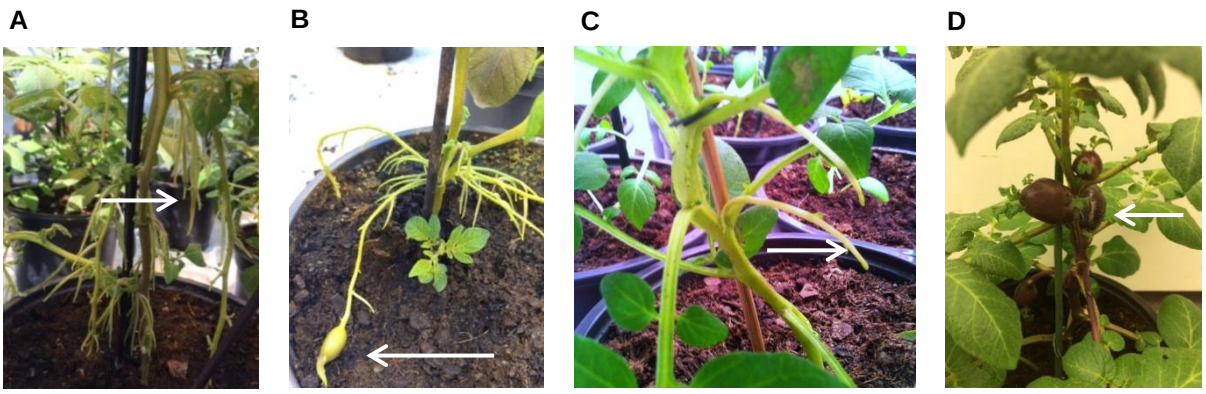

Figure 8.

SUPPLEMENTARY DATA

Table S1. Overview of the five experiments testing how tuberization affects potato flower bud development.

Exp.	Method of tuber removal	Plants per treatment	Genotypes	**Conditions:** day-length (hours), day/night temperature (°C), light intensity (µmol·s^{-1}·m^{-2}), relative humidity, duration (weeks), location
1a	**Removing tuber-sink**: Grafting	10	CE3027 *S. etuberosum* *S. palustre*	8, 22/18**, not measured (winter-spring 2016), not measured, 14, greenhouse
1b	**Removing tuber-sink**: Grafting	10*	CE3027 CE3130 *S. etuberosum*	16-17, 22/18**, not measured (summer 2016), not measured, 12, greenhouse
2	**Removing tuber-sink**: Removing stolons	11	CE3027	8 & 16, 20/20, 200 and 400, 70%, 8, climate chamber
3	**Removing tuber signal**: Silencing *StSP6A*	8 5 5	*S. andigena* WT *StSP6A RNAi#1* *StSP6A RNAi#13*	8 & 16, 22/18, 200 and 400, 70%, 8, climate chamber
4	**Removing tuber signal and sink:** Silencing *StSP6A* and removing stolons	10	*S. andigena* *StSP6A RNAi#13*	8, 22/18, 400, 70%, 8, climate chamber
5	**Increasing the tuber signal:** Overexpressing *StCDF1*	10 8 8	CE3027 *35S::StCDF1 #3* *35S::StCDF1 #4*	16, 20/18, 200, 70%, 6, climate chamber

* Some grafting combinations exceeded or failed to reach this number (*n* indicated in the results)

** In the greenhouse in the daytime temperatures sometimes exceeded the set temperature of 22ºC.

Table S2. Flower bud development and tuberization in grafts between CE3130 and *S. etuberosum*.

A	Rootstock	*n*	Anthesis *	Maximum open flowers/plant	Tuber dry weight (g/plant)
Scion CE3130	CE3130 (tuberizing)	10	9	2.6 ±1.4 b**	8.0 ±1.1 b
	S. etuberosum (non-tuberizing)	9	1	0.4 ±1.3 a	0.0 ±0.0 a

B	Rootstock	*n*	Anthesis	Maximum open flowers/plant	Tuber dry weight (g/plant)
Scion *S. etuberosum*	*S. etuberosum* (non-tuberizing)	3	1	3.3 ±3.1 a	0.0 ±0.0 a
	CE3130 (tuberizing)	11	4	3.0 ±3.0 a	2.3 ±1.5 a
	CE3130 + leaves (tuberizing)	12	5	3.8 ±2.6 a	6.5 ±2.3 b

* Plants that reached the open flower stage

** Standard deviations are given, identical alphabetical letters indicate no significant difference between graft combinations ($\alpha = 0.05$)

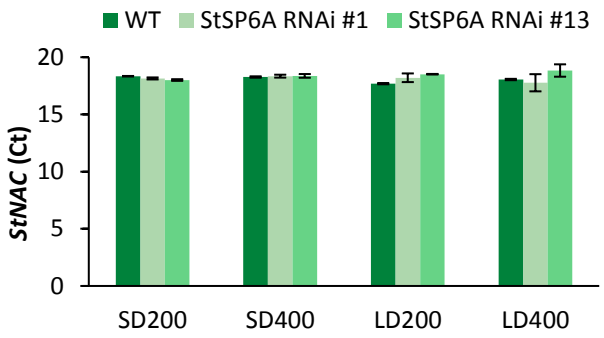

Figure S1. *StNAC* expression (Ct = cycle threshold) in wild-type *S. andigena*, *StSP6A RNAi* #1, and *StSP6A RNAi* #13. The reference gene *StNAC* is similarly expressed in all tested light treatments and genotypes. Plants were grown in short days (SD) of 200 and 400 µmol·m^{-2}·s^{-1} and long days (LD) of 200 and 400 µmol·m^{-2}·s^{-1}. The error bars show the standard deviation within a treatment/genotype. Technical repetitions, n = 3.

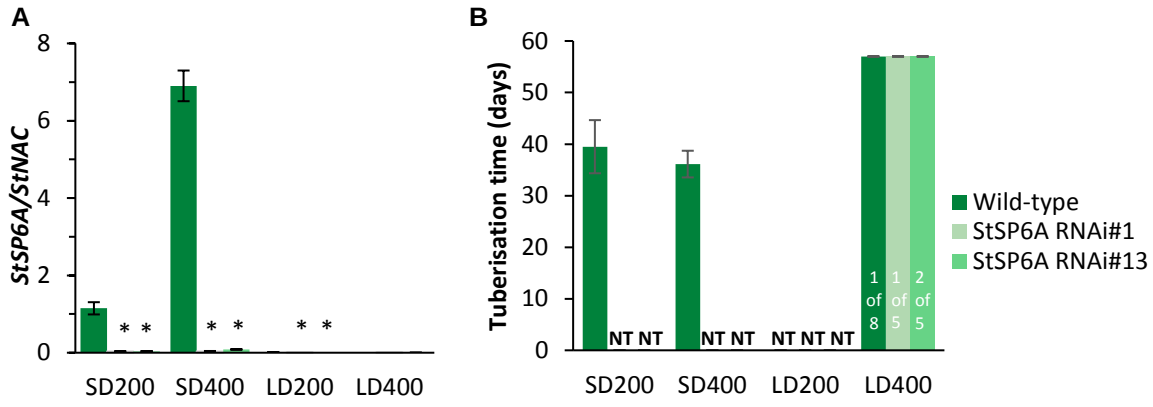

Figure S2. *StSP6A* expression and tuberization time in wild-type *S. andigena*, *StSP6A RNAi* #1, and *StSP6A RNAi* #13. Plants were grown in short days (SD) of 200 and 400 µmol·m^{-2}·s^{-1} and long days (LD) of 200 and 400 µmol·m^{-2}·s^{-1}. (A) *StSP6A* is expressed relative to the reference gene *StNAC*. Expression is from plants five weeks after transplanting. The error bars show the standard deviation. Asterisks indicate a significant difference to the wild-type expression in a given light treatment ($\alpha = 0.05$). Technical repetitions, $n = 3$. (B) Tuberization time in days from transplanting to soil. The error bars show the standard deviation. The fraction of tuberizing plants is indicated in the bar (no indication means all plants tuberized). Biological replicates: Wild-type ($n = 8$), *StSP6A RNAi* #1 ($n = 5$), *StSP6A RNAi* #13 ($n = 5$). (Exp. 3).