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# The tuberization signal StSP6A represses flower bud development in potato

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35 **TITLE**

37 **The tuberization signal StSP6A represses flower bud development in potato**

39 **Running title: Tuberization signal represses potato flower development**

42 **HIGHLIGHT**

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

48 **ABSTRACT**

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

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

69

70 **INTRODUCTION**

71

72 Potato (*Solanum tuberosum* L.*)* is the third largest crop for human consumption worldwide and
73 due to its high nutritional value and low production costs, consumption is most certainly
74 expected to increase (International Potato Center, 2016; Zaheer and Akhtar, 2016). Potato
75 plants are able to reproduce both sexually, through flowers, and asexually through the
76 formation of tubers. Although both reproduction methods are present in the plant, most research
77 has been done on tuberization. Commercial potato production mainly uses "seed tubers" and
78 not "true seeds" to propagate plants. Asexual reproduction is used for propagation because
79 potato plants are tetraploid and highly heterozygous. Incorporation of a *Sli* gene allows for self-
80 fertilization of diploid potato lines, which makes the generation of homozygous lines possible
81 (Lindhout *et al*., 2011). These developments have made hybrid breeding in potato possible and
82 thereby also the use of true potato seeds as starting material. Hybrid breeding of potato will
83 enable breeders to specifically select for desired traits in new varieties and develop these
84 varieties much faster than in traditional potato breeding (Lindhout *et al*., 2011). The
85 developments in potato breeding and propagation require the understanding of not only
86 tuberization, but also potato flowering.
87 Whether a potato plant starts to tuberize or flower, depends strongly on environmental cues
88 (Ewing and Struik, 1992; Almekinders and Struik, 1996). Potato tuberization is strongly
89 influenced by day length and is induced under short-day conditions (Batutis and Ewing, 1982).
90 Modern varieties are no longer dependent on short days to tuberize, as breeders have selected
91 against this trait. Nevertheless, the photoperiodic mechanism controlling tuberization remains
92 conserved in all potato plants (Kloosterman *et al*., 2013). As potato tuberization has been
93 intensively studied, we have a good understanding of the molecular regulation behind this
94 process (Abelenda *et al.* 2011; Navarro *et al.*, 2011; González-Schain *et al.* 2012; Navarro *et*
95 *al.*, 2015). The photoperiodic regulation of tuberization strongly resembles the photoperiodic
96 control of flowering time in the model plant *Arabidopsis thaliana* and other plants (Tsuji *et al.,*
97 2011; Andrés and Coupland, 2012; Fu *et al.*, 2014). *SELF-PRUNING 6A* (*StSP6A*) was
98 identified as a potato homologue of the flowering signal *FLOWERING LOCUS T* (*FT*) in *A.*
99 *thaliana* and instead of inducing the flower transition, StSP6A induces tuber formation in
100 potato (Potato Genome Sequencing Consortium, 2011; Navarro *et al.*, 2011). After *StSP6A* is
101 expressed in the leaves, the mobile StSP6A protein moves through the plant to underground
102 stems, called stolons, where it induces tuberization. The cascade of events leading to short-day

103 dependent expression of *StSP6A* mRNA has also been revealed. This control includes the genes
104 *CYCLING DOF FACTOR* (*StCDF1*) and *CONSTANS* (*StCO*) (Kloosterman *et al*., 2013),
105 which are also involved in photoperiodic control of flowering in *A. thaliana*. In potato StCDF1
106 downregulates *StCO*, which in turn induces *SELF-PRUNING 5G* (*StSP5G),* a repressor of
107 *StSP6A* (Kloosterman *et al*., 2013; Abelenda *et al*., 2016). Within the Solanaceae, the *FT*
108 family has undergone a large expansion and another homologue of *FT* called *SELF-PRUNING*
109 *3D* (*StSP3D*) was found in potato and was proposed to control the flower transition (Potato
110 Genome Sequencing Consortium, 2011; Navarro *et al*., 2011). However, how this regulation
111 takes place remains to be elucidated.
112 Although some research has been performed on potato flowering, ambiguity remains
113 concerning the environmental effect on flower transition and whether this is a long-day, short-
114 day or day-neutral processes (Jones and Borthwick, 1938; Almekinders and Struik, 1994;
115 Navarro *et al*., 2011; González-Schain *et al*., 2012). Although little is known about the flower
116 transition, it has been established that potato flower development is negatively affected in tuber
117 inducing conditions like short days (Turner and Ewing, 1988; Rodríguez-Falcón *et al*., 2006;
118 Plantenga *et al*., 2016). Flower buds abort more frequently and less open flowers are formed.
119 Failure of flower bud development in short days could be due to a direct photoperiod effect,
120 but alternatively might be the result of a negative effect exerted by tuberization. Tubers are
121 strong assimilate sinks (Sweetlove *et al.,* 1998) and may leave insufficient assimilates to
122 support flowering (Almekinders and Struik, 1996). However, previous studies do not agree
123 whether or not flowering competes with tuberization (Krantz, 1939; Thijn, 1954; Jessup, 1958;
124 Krauss and Marschner, 1984; Pallais, 1987).
125 Here we confirm that while the flower transition occurs independently of photoperiods, later
126 stages of flower bud development are impaired under short-day conditions which induce
127 tuberization. Specifically, we investigated whether flower bud development is impeded by
128 competition for assimilates between flowering and tuberization or by the tuberization signal
129 StSP6A. We performed experiments where we prevented tuberization in three different ways;
130 (1) by grafting potato scions onto wild potato rootstocks, that were unable to form tubers; (2)
131 by removing stolons, the structures on which tubers form; (3) by using transgenic plants that
132 were silenced in the tuberization signal *StSP6A* (Fig. 1). Finally we demonstrated how
133 increased *StSP6A* expression affected flower bud development in long days. Together, our
134 experiments show that the tuberization signal StSP6A inhibits flower bud development and
135 only the repression of this signal improves flower bud development.
136

## 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

171 *et al*., 2013). Binary plasmids were obtained after LR clonase$^{TM}$ II enzyme (Invitrogen)
172 reaction of StCDF1.1-pENTR™/D-TOPO® with the pK7WG2 plasmid, obtaining the
173 35S::StCDF1.1 plasmid (Karimi et al., 2002). In order to generate 35S::StCDF1 transgenic
174 plants, *Agrobacterium*-mediated transformation of CE3027 internodes with both plasmids was
175 performed as described in Visser (1991).
176 *S. andigena StSP6A RNAi* and CE3027 *35S::StCDF1* plantlets were propagated *in vitro* and
177 grown with the other potato plants.
178

179 **Growing conditions and measurements**

180 *Exp.1. Removing the tuber-sink: grafting onto a non-tuberizing rootstock*
181 Two grafting experiments were performed in a greenhouse in short- and in long-day conditions.
182 In short days CE3027 scions were grafted onto *S. etuberosum* and *S. palustre* rootstocks and
183 vice versa. Also control grafts were made where scions were grafted onto rootstocks of their
184 own genotype. In long days, nine grafting combinations were made between CE3027, CE3130
185 and *S. etuberosum*. CE3027 and CE3130 scions were grafted onto *S. etuberosum* rootstocks, *S.*
186 *etuberosum* scions were grafted onto CE3027 and CE3130 rootstocks, and control grafts were
187 made with scions and rootstocks from the same genotype. Additionally, *S. etuberosum* scions
188 were grafted onto CE3027 and CE3130 rootstocks, which maintained their leaves, to ensure
189 the production of the tuberization signal StSP6A (climate details in Supplementary Table S1).
190 *In vitro* plantlets were transplanted to 5 L pots with a clay-peat mixture. Grafting was done
191 with two-week old CE3027 and CE3130 plants and three-week old *S. etuberosum* and *S.*
192 *palustre* plants. The stem was cut after the fourth leaf from the bottom. A splice-graft was made
193 and the rootstock and scion were kept together with silicone grafting clips (Beekenkamp 1.5
194 mm and Simonetti 2.9 mm). Leaves were removed from the rootstock, unless indicated
195 otherwise. Grafts were placed in a high humidity compartment until the grafting unions had
196 set. The plants were manually watered and fertilized ($2g \cdot L^{-1}$, Osmocote Exact Standard 3-4M,
197 Everris). Flowering and tuberization was determined once a week. Anthesis (opening flowering
198 stage) of the primary stem and the maximum number of open flowers per plant were noted.
199 Tuberization time was determined by carefully checking the stolon tip for swelling. Nine weeks
200 after grafting, the tubers were harvested, oven-dried at 105ºC and weighed.
201 *Exp. 2. Removing the tuber-sink: removing stolons*
202 CE3027 plantlets were transplanted to 17cm Ø pots with a clay-peat mixture and placed in a
203 climate chamber (details in Supplementary Table S1). Plants were grown in short days (8 hours
204 light) under 200 or 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (photosynthetic photon flux density) light (SD200 and

205 SD400 respectively) and in long days (16 hours light) under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light (LD200).
206 The high-light short day and low-light long day received the same daily light sum. In half of
207 the plants in each light treatment, stolons were removed as soon as formed, resulting in six
208 treatments in total. Light emitting diodes (LEDs) were used for the lighting (Philips
209 GreenPower LED production module 120 cm DeepRed/White-2012). Light intensities were
210 measured at the top of the plant canopy with a quantum sensor (LI-COR Biosciences, LI-190SB
211 Quantum, LI-1400 data logger) and corrected by adjusting LED height every two weeks. Plants
212 were rotated three times a week to ensure a homogenous light distribution. Side-shoots were
213 removed. Water was given manually and liquid fertilizer was supplied once per week (EC 2.1
214 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
215 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
216 $MoO_4^{2-}$). Plants were examined three times a week for stolons, flower bud appearance, anthesis,
217 number of flowers and tuberization. A destructive harvest including fresh and dry weight
218 measurements of tubers and shoot (aboveground stem, leaves and shoot apex) was done after
219 eight weeks.
220 *Exp. 3. Removing the tuberization signal: reducing StSP6A expression*
221 Plants of *S. andigena* wild-type and two *StSP6A RNAi* lines (#1 and #13) were transplanted to
222 17cm Ø pots and placed in a climate chamber (details in Supplementary Table S1). In addition
223 to the three light treatments used in Exp. 2, a long-day treatment of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LD400)
224 was applied. Plants were grown and examined as in Exp. 2. Additionally, flower bud
225 development was recorded (flower bud size was categorized from zero to five where zero was
226 no flower bud and five was an open flower). This was done due to the bad flowering success
227 of *S. andigena* and the low chances of reaching anthesis. A destructive harvest was performed
228 after eight weeks of growing and included fresh and dry weight measurements of tubers and
229 shoot.
230 *Exp. 4. Removing the tuberization signal and tuber sink: reducing StSP6A expression and*
231 *removing stolons*
232 *S. andigena* wild-type and *StSP6A RNAi* #13 plants were transplanted to 17cm Ø pots and
233 placed in a short-day chamber with 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light from fluorescent tubes (Philips;
234 Master TL-D Reflex 58W/840 Coolwhite) (climate details in Supplementary Table S1). In half
235 of the wild-type *S. andigena* plants, stolons were removed. Stolons were also removed in half
236 of the *StSP6A RNAi* #13 plants to determine whether stolon removal affected plant growth in
237 non-tuberizing plants. Plant growth control and determination of tuberization time and flower
238 bud appearance were performed as in Exp. 2 and 3. Because flower bud size was only

239 categorized and not measured precisely in Exp. 3, flower bud development in Exp. 4 was
240 determined by measuring the diameter of the biggest flower bud on each plant, three times a
241 week.
242 *Exp. 5. Increasing the tuberization signal: overexpressing StCDF1 in long days*
243 An additional experiment was performed to confirm that StSP6A affected flower bud
244 development. Instead of reducing *StSP6A* in short days, *StCDF1* overexpressing lines were
245 used with upregulated *StSP6A* expression in long days. Eight wild-type CE3027, eight
246 *35S::StCDF1#3* and eight *35S::StCDF1 #4* plantlets were transferred to 15 cm Ø pots and
247 placed in a long-day chamber with 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light from fluorescent tubes (Philips;
248 Master TL-D Reflex 58W/840 Coolwhite) (details climate in Supplementary Table S1). Plant
249 growth control was performed as in Exp. 2, 3 and 4. Photographs of the shoot apex were taken
250 after eight weeks of growing and anthesis was documented.
251
252 **RNA analysis**
253 *StSP6A* expression was analyzed to determine if the *StSP6A* silenced lines were indeed silenced
254 in *StSP6A* and if the *StCDF1* overexpressing lines had upregulated *StSP6A*. Furthermore,
255 *StSP3D* expression was analyzed to determine if the *StSP6A* silenced lines did not increase
256 expression of the flowering signal *StSP3D*. Leaf samples of the plants in Exp. 3 were collected
257 after five weeks, just before the first tuberization started. The fifth leaf from the top was
258 sampled one hour after the lights went on. Leaves from three plants were collected, pooled into
259 one sample and frozen in liquid nitrogen and stored at -80ºC. Leaves were also collected from
260 Exp. 5. The fourth and fifth leaf from the top were collected after five weeks, two hours after
261 lights went on. Two plants were pooled and four pools per genotype were made. Gene
262 expression was determined using qPCR (quantitative reverse transcription polymerase chain
263 reaction). Frozen leaf material was ground and used for RNA extraction with an RNeasy plant
264 mini-kit (Qiagen). A spectrophotometer (NanoDrop, Thermoscientific, Thermofisher)
265 determined RNA concentration and quality. A DNase treatment was performed using
266 Amplification grade DNase I (Invitrogen, Thermofisher). 1µg of RNA was used for cDNA
267 synthesis with an iScript kit (Bio-rad). RNA extraction, DNase treatment and cDNA synthesis
268 were performed as described in the supplied manufacturer's protocols. 20µl of cDNA was
269 diluted to a total volume of 150 µl. 5µl of SYBR-green (iQ-SYBR-green super mix, Bio-Rad),
270 0.25µl Forward Primer (10µM), 0.25µl Reverse Primer (10µM), 0.5µl Milli-Q water and 4µl
271 diluted cDNA were used for the qPCR. In Exp. 3 three technical replicates were used per pooled
272 sample. Samples were placed in a Thermal Cycler (C1000, Bio-Rad) set to 95°C for 3 minutes,

273 40 cycles of 95°C for 15 seconds and 60°C for 1 minute, followed by 95°C for 10 seconds and
274 for a melt curve 65°C to 95°C in 0.5°C steps every 5 seconds. Primers used were: *StSP6A*
275 (PGSC0003DMT400060057): (F) GACGATCTTCGCAACTTTTACA, (R)
276 CCTCAAGTTAGGGTCGCTTG and *StSP3D* (scaffold PGSC0003DMB00000014,
277 unannotated): (F) GGACCCAGATGCTCCAAGTC, (R) CTTGCCAAAACTTGAACCTG
278 and *StNAC* (reference gene *NASCENT POLYPEPTIDE-ASSOCIATED COMPLEX ALPHA*,
279 PGSC0003DMT400072220): (F) ATATAGAGCTGGTGATGACT, (R)
280 TCCATGATAGCAGAGACTA. Primers for *StSP6A* and *StSP3D* were used in (Navarro et al.,
281 2011) and the *StNAC* primer had an efficiency of 99%.
282
283 **Data analysis**
284 A student's t-test was used to compare two treatments and a one-way analysis of variance
285 (ANOVA) was used to compare more than two treatments. A Bonferonni pair-wise comparison
286 was used to determine which treatments significantly differed ($\alpha = 0.05$, IBM, SPSS Statistics
287 22 and GenStat, 18th Edition). When data was ordinal or not normally distributed (tested with
288 a Shapiro-Wilk W-test for non-normality in GenStat), a non-parametric Kruskal-Wallis test
289 and Dunn's pairwise comparisons ($\alpha = 0.05$) were computed in SPSS. Comparisons between
290 light treatments in Exp. 2 and 3 were based on biological replicates as independent
291 experimental units. For gene expression analysis three technical replicates were used for the
292 qPCR analysis in Exp. 4 and four biological replicates were used for qPCR analysis in Exp. 5.
293 $100/2^{-\Delta Ct}$ was used to determine gene expression values. Ct (cycle threshold) values of the gene
294 of interest (*StSP6A* and *StSP3D*) were used to determine expression of the gene of interest
295 compared to the housekeeping gene *StNAC*. Invariant expression of *StNAC* under the tested
296 conditions is shown in Supplementary Fig. S1.
297
298
299 **RESULTS**
300
301 **Removing the tuber-sink: grafting onto a non-tuberizing rootstock**
302 In order to establish how the absence or presence of tubers would affect flowering of the scions,
303 two grafting experiments were performed in short-day and long-day conditions.
304
305 *Grafting under short day conditions*

Short-day conditions strongly promote tuberization. To determine whether flower bud development in CE3027 would improve without tubers, we grafted CE3027 scions onto non-tuberizing *S. etuberosum* and *S. palustre* rootstocks and grew them in short-day conditions. CE3027 scions underwent floral transition and as expected the flower buds failed to develop in the control grafts with tuberizing CE3027 rootstocks. Moreover, the buds also failed to develop when the CE3027 scion was grafted onto the non-tuberizing *S. etuberosum* or *S. palustre* rootstocks. Thus, the absence of tubers could not improve flower bud development. Opposite grafts were made with *S. etuberosum* and *S. palustre* scions on CE3027 rootstocks to determine how tubers would affect flower bud development. However, in the short-day conditions, neither *S. etuberosum* nor *S. palustre* transitioned to flowering and the CE3027 rootstocks failed to tuberize. To gain a better understanding on the effect of tubers on flower bud development, and attempt to induce flowering in *S. etuberosum,* a grafting experiment was performed under long-day conditions.

*Grafting under long day conditions*

Grafting CE3027 scions onto non-tuberizing *S. etuberosum* rootstocks did not improve flower bud development compared to the control grafts, which tuberized (CE3027 scion on CE3027 rootstock) (Fig. 2). Surprisingly, the opposite effect was observed. Although all tested graft combinations underwent flower transition (data not shown), grafts with CE3027 scions on *S. etuberosum* rootstocks only reached anthesis in four of the eight (50%) plants, whereas nine of the eleven (>80%) control grafts reached anthesis (Fig. 3). Furthermore, the grafts with *S. etuberosum* rootstocks had almost half the number of open flowers compared to control grafts. The grafts made with scions of the early tuberizing genotype CE3130 also had impaired flower bud development in grafts with non-tuberizing rootstocks (Supplementary Table S2A).

To determine if the presence of tubers would impair *S. etuberosum* flower bud development, we made opposite grafts with *S. etuberosum* scions on tuberizing CE3027 rootstocks. In contrast to the short-day grafting experiment, flower transition occurred in *S. etuberosum* and the flower buds developed into open flowers. Furthermore, CE3027 rootstocks tuberized, even when *S. etuberosum* scions were grafted onto them. However, a larger fraction of grafts with *S. etuberosum* scions on tuberizing CE3027 rootstocks reached anthesis, than of control grafts with *S. etuberosum* scions on non-tuberizing *S. etuberosum* rootstocks (Table 1). When comparing grafts in which the CE3027 rootstock was completely defoliated, with grafts in which some leaves were kept below the graft junction, the presence of leaves accelerated tuberization in CE3027 rootstocks with approximately nine days (data not shown). Also, a

340 larger fraction of grafts with leafy CE3027 rootstocks reached anthesis than grafts with leafless
341 CE3027 rootstocks. Grafts with *S. etuberosum* scions on the early tuberizing CE3130
342 rootstocks showed a similar result. Anthesis was higher in grafts with CE3130 rootstocks and
343 the presence of leaves accelerated tuberization, and also increased the number of plants with
344 open flowers. (SupplementaryTable S2B). Thus, tuberizing rootstocks did not impair the
345 flower bud development of *S. etuberosum* scions.
346 Taken together, the interspecific grafting experiments did not show that the presence of tuber
347 sinks impaired flower bud development, but rather had an unexpected opposite outcome where
348 an improved flower bud development was observed in grafts producing tubers. To validate that
349 these results were not due to interspecific interaction in the grafts, we performed another
350 experiment where the tuber-sink was removed within the same genotype.
351
352 **Removing the tuber-sink: removing stolons**
353 To determine whether tubers negatively influenced flower bud development, tuberization was
354 prevented by removing the stolons in CE3027 plants. Removing stolons did not significantly
355 affect the number of flowering plants nor the number of open flowers per plant (Table 2). Also,
356 the time until anthesis was not affected by removing the stolons (data not shown). The light
357 conditions under which plants were grown did affect flower bud development. The number of
358 flowering plants was low in low-light short days (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$), but in high-light short days
359 (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) the number of flowering plants was almost as high as in long days (200
360 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Nevertheless, removing stolons did not improve flower bud development, both
361 under short-day and long-day conditions.
362 These results confirm that short-day conditions impair flower bud development in CE3027.
363 Although the number of flowering plants was similar in a high-light short day compared to a
364 low-light long day (SD400 and LD200 had the same daily light sum), the maximum number
365 of open flowers per plant was significantly higher in the long day treatment (1.2 flowers in
366 SD400 vs. 6.5 in LD200).
367 Tuberization took place in all light treatments unless stolons were removed (Table 2). The
368 short-day treatment with high light intensity resulted in the fastest tuberization and the highest
369 tuber biomass. Plants without stolons had a higher shoot biomass than plants with stolons. The
370 light treatments with the highest light sum (SD400 and LD200) had a higher shoot biomass
371 than the low light sum short day (SD200), in both tuberizing and non-tuberizing plants. The
372 total biomass (tuber + shoot) was highest in the high-light short-day treatment.

373 In summary, preventing tuberization by removing the stolons did not improve flower bud
374 development, even though flower bud development was impaired in short days.
375
376 **Removing the tuberization signal: reducing *StSP6A* expression**
377 To determine whether the tuberization signal negatively influenced flower bud development,
378 we used transgenic plants with reduced expression of the tuberization signal *StSP6A*. Flower
379 buds were formed in all *S. andigena* plants, but flower bud development of the *S. andigena*
380 wild type was impaired in short days compared to long days (Fig. 4). Wild-type plants under
381 high-light short-day and low-light long-day conditions (SD400 and LD200) received the same
382 daily light sum, but flower buds were smaller in the short-day treatment (Fig. 5A, $P = 0.02$). In
383 the *StSP6A RNAi* lines grown under short-day conditions, flower bud development was
384 improved compared to the wild type. Under these conditions, flower bud development in the
385 *StSP6A RNAi* lines equaled the level of flower bud development in the wild-type plants under
386 long-day conditions (Fig 5A, no significant difference between short day *StSP6A RNAi* lines
387 and long day wild-type lines, $P = 0.12$). Moreover, two of the five *StSP6A RNAi* #13 plants in
388 the high-light short days reached anthesis, which did not occur in *S. andigena* wild-type or
389 *StSP6A RNAi* plants in any other treatment, not even in long days (Fig. 5B). In long days, a
390 lower *StSP6A* expression did not have an effect on flower bud development. Reducing *StSP6A*
391 expression did not affect the flower transition time in either short or long days. Gene expression
392 analysis of *StSP6A* in *StSP6A RNAi* lines show that these lines were indeed silenced in *StSP6A*
393 (Supplementary Fig. S2A). As expected, tuberization in the transgenic lines with reduced
394 *StSP6A* expression, was inhibited compared to the wild-type plants (Supplementary Fig. S2B).
395 Wild-type plants in high-light long-day conditions showed a later and reduced tuberization
396 compared to the plants in short days, while low-light long-day plants did not tuberize at all. In
397 summary, our results show that inhibiting tuberization by reducing *StSP6A* expression in potato
398 plants grown under short-day conditions improves flower bud development.
399
400 **Removing the tuberization signal and the tuber-sink: reducing *StSP6A* expression and**
401 **removing stolons**
402 As the experiments testing the removal of the tuber-sink used different genotypes than the
403 experiments testing removal of the tuberization signal *StSP6A*, we performed a short-day
404 experiment with *S. andigena*, where the tuberization signal *StSP6A* and the stolons were
405 removed. Also, flower bud development was measured in more detail, to better illustrate
406 differences between treatments. Plants with reduced levels of *StSP6A*, clearly developed larger

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

413

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

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

421

422 **All experiments: plant growth after removing the tuber-sink**

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

434

435

436 **DISCUSSION**

437

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

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

441 reached (Fig. 3A-B). This is in line with results in opposite grafts, where the effect of tuberizing
442 rootstocks on *S. etuberosum* scions was tested; in these grafts the fraction of plants with open
443 flowers increased compared to control grafts with *S. etuberosum* rootstocks (Table 1). The
444 results show that removing the tuber-sink does not improve flower bud development.
445 That tuberizing rootstocks did not impair, but improved flower development in *S. etuberosum*
446 scions, was surprising. Instead of inhibiting flower development, tuberization may improve
447 flowering in a different species (*S. etuberosum*). The FT of one species can induce flowering
448 or tuberization in another species, for instance with rice *Heading date 3a* (*Hd3a)* in potato,
449 *Arabidopsis FT* in tomato and tobacco, and tomato *SINGLE FLOWER TRUSS (SFT)* in
450 Arabidopsis (Lifschitz *et al*., 2006; Lifschitz and Eshed, 2006; Navarro *et al*., 2011). Potato
451 StSP6A from the rootstock may improve *S. etuberosum* flowering in the scion. Interestingly,
452 long-day grafts between *S. etuberosum* scions and leafless CE3027 or CE3130 rootstocks
453 flowered and tuberized, while short-day grafts between *S. etuberosum* scions and the leafless
454 CE3027 and CE3130 rootstocks did not (data not shown). Perhaps in long days, a leaf-derived
455 FT from *S. etuberosum* induces tuberization, while in short days this signal is not produced.
456 Potato plants are thought to have an auto regulatory *StSP6A* loop, where leaf-derived StSP6A
457 leads to upregulation of *StSP6A* in the stolons, enhancing the level of StSP6A for tuberization
458 (Navarro *et al*., 2011). Potato scions expressing rice *Hd3a* but no *StSP6A* have induced *StSP6A*
459 in the stolons (Navarro *et al*., 2011). If FT from *S. etuberosum* also induces this auto regulatory
460 loop, *S. etuberosum* FT may induce tuberization in the CE rootstocks and amplify the amount
461 of FTs in the graft, possibly enhancing flowering as well.
462 Most importantly our grafting experiments show that the tuber-sink does not impair flower bud
463 development. However, because interspecific grafts were used, effects on flowering may have
464 been caused by other properties of the *S. etuberosum* than its inability to tuberize. Therefore,
465 to determine whether removing the tuber-sink improves flower bud development, stolons were
466 removed in potato plants.
467
468 *Removing stolons did not improve flower bud development*
469 As with grafting, removing the stolons did not improve flower bud development in both
470 CE3027 and *S. andigena* genotypes (Table 2 and Fig. 6). This is in line with previous
471 experiments on stolon abscission (Weinheimer and Woodbury, 1966). Removing stolons also
472 had no effect on flower initiation. The lack of stolons did lead to an increase of assimilates
473 available for the shoot, as seen in the significant increase in shoot biomass (Table 2). However,
474 this increase in shoot biomass did not improve flower bud development.

475 In short days, flower bud development was impaired compared to long days (Table 2).
476 However, by raising short-day light intensity to match the light sum of long days, the fraction
477 of plants to reach anthesis increased from two out of eleven plants (< 20%) to ten out of eleven
478 plants (> 90%), which almost rivalled long-day anthesis (anthesis in all plants). Sufficient light
479 is crucial for flower bud development, as has been demonstrated in several crops including
480 potato and tomato (Kinet, 1977; Demagante and Zaag, 1988; Turner and Ewing, 1988).
481 Increasing light may increase the amount of assimilates formed in the plant. Assimilates like
482 sucrose play an important role in flower induction and floral development in potato (Chincinska
483 *et al.*, 2008). Nevertheless, the number of open flowers was significantly higher under long-
484 day conditions, indicating an impairment of CE3027 flowering in short days, as was found
485 before in other potato genotypes (Turner and Ewing, 1988). Thus, short-day flower bud
486 development was impaired and preventing formation of the tuber-sink by removal of the
487 stolons did not improve this development.
488
489 *Removing the tuber-sink in a plant that was induced to tuberize led to "aerial stolons"*
490 Removing the tubers did not improve flower bud development. Although the plants were
491 unable to tuberize, they were still induced to do so. Grafts that could not tuberize, started to
492 produce stolon and tuber-like structures on the scions (Fig. 8A-B). Plants without stolons,
493 growing in short days, also made stolon-like structures on the stem (Fig. 8C-D). Alternative
494 tuberization structures have been documented before (Thijn, 1954; Weinheimer and Woodbury,
495 1966) in conditions where tuberization is prevented but plants remain induced to tuberize. The
496 lack of tubers led to more assimilates in the shoot, but instead of promoting flowering these
497 assimilates may have been directed towards alternative tuberization structures. The
498 tuberization signal *StSP6A* is still expressed in inducing conditions, even when tubers are
499 removed, which may be the cause of the direction of assimilates to alternative tuberization
500 structures instead of to the flower buds. This theory is supported by the finding that the
501 formation of stolon-like structures in short-day *StSP6A RNAi* plants was much less severe.
502
503 *The tuberization signal StSP6A impairs flower bud development*
504 *S. andigena* wild-type plants underwent floral transition in all tested light treatments, but in
505 short days the flower buds ceased to develop at a very early stage (Fig. 5A). Remarkably, short-
506 day flower bud development was significantly improved in the *StSP6A RNAi* lines. Two of the
507 *StSP6A RNAi* plants were even able to reach anthesis in short days, which did not happen in
508 any other treatment and is uncommon for *S. andigena* when grown in our climate chamber

509 conditions. Flowering in *StSP6A RNAi* plants was also tested by Navarro *et al.* (2011), but only
510 transition to flowering was considered and not flower bud development. The transition to
511 flowering occurred at the same time as in the wild type, as was the case in our experiments
512 (data not shown). The transgenic lines had a significantly reduced *StSP6A* expression
513 (Supplementary Fig. S2A). Improved flower bud development in the transgenic lines could not
514 be explained by an increase in transcription of the proposed flowering signal *StSP3D* in the
515 leaves (data not shown), implying that *StSP6A* negatively affects flower bud development
516 through a different mechanism.
517 StSP3D has been proposed to be the flowering signal in potato, because silencing *StSP3D*
518 showed a late flowering response (Navarro *et al*., 2011). However, there is a lack of correlation
519 between flower bud development and *StSP3D* expression, which is strongly expressed under
520 short day conditions but weakly expressed under long day conditions (in prep. Dr. S. Bergonzi).
521 Perhaps low expression levels of *StSP3D* are sufficient to induce flowering and the level of
522 *StSP6A* determines the success of flower bud development. To fully understand potato
523 flowering, elucidating the role of StSP6A in flower bud development, as well as StSP3D in
524 flowering time and development, will be crucial.
525 Our finding that StSP6A represses flower bud development, while the tuber-sink does not, was
526 confirmed in another experiment testing both stolon abscission (tuber-sink) and silencing of
527 *StSP6A* (tuberization signal) in *S. andigena* in short days. Removal of stolons did not improve
528 flower bud development, while downregulation of *StSP6A* did. The repressing role of StSP6A
529 on flower bud development was further confirmed in CE3027 *StCDF1* overexpressing lines,
530 with upregulated *StSP6A* in long days. The flower bud development was impaired in these lines
531 and resembled the impaired flower bud development found in wild-type *S. andigena* plants in
532 short days. Transgenic lines in which an upstream regulator of *StSP6A* was overexpressed were
533 used instead of *StSP6A* overexpressing lines, to induce *StSP6A* in long days. In Navarro (2011),
534 transgenic lines overexpressing *StSP6A* actually improved flowering, perhaps by the strong
535 and ubiquitous expression of *StSP6A* by the 35S promotor (Odell *et al.,* 1985; Seternes *et al.*,
536 2016). In the *StCDF1* overexpressing lines, the down-stream regulation on *StSP6A* is still intact,
537 allowing a more realistic upregulation of *StSP6A* than in a *35S::StSP6A* overexpressing line.
538 Flower impairment in these lines confirms our earlier findings that StSP6A represses flower
539 bud development.
540
541 *Can the inhibiting effect of a tuber-sink be ruled out?*

542 Although flower bud development was not improved by tuber-sink removal in CE3027,
543 CE3130 or *S. andigena*, removing the tuber-sink had a positive effect on flowering in some
544 genotypes in the past (Thijn, 1954; Jessup, 1958). However, these reports have also been
545 contradicted (Turner and Ewing, 1988). Therefore, it may be possible that repression of flower
546 development by the tuber-sink is genotype specific. It would be interesting to find out if
547 reducing *StSP6A* would further improve flower development in genotypes that are benefitted
548 by tuber-sink removal. Nevertheless, our findings show that in *S. andigena* and CE3027 the
549 tuber-sink does not repress flower bud development while the tuberization signal StSP6A does.
550
551 *The day-length control of flowering in potato*
552 Short days, or more correctly long nights, induce tuberization in potatoes, although variation
553 exists between varieties in their dependence on short days (Garner and Allard, 1923; Ewing
554 and Struik, 1992; Prat, 2010; Kloosterman *et al.*, 2013). Potato flowering has been categorized
555 as a short-day, long-day and day-neutral process (Jones and Borthwick, 1938; Turner and
556 Ewing, 1988; Almekinders and Struik, 1994; Martínez-García *et al.*, 2002; Schittenhelm *et al.*,
557 2004). A cause for this variation might be the use of different genotypes and the difference in
558 defining flowering. Because flowering is a process composed of many phases, it needs a clear
559 distinction when addressed: it starts with flower transition and proceeds with flower bud and
560 organ development. More importantly, the flowering process is not only influenced by day
561 length but by tuberization as well, which varies between genotypes. Our results show that the
562 floral transition occurs independently of the photoperiod but that flower bud development is
563 repressed by the tuberization signal. Remarkably anthesis was only attained in short days with
564 high irradiance (in *StSP6A RNAi*#13) indicating that environmental growing conditions also
565 play a role in the process. Our results point to a short-day control of flower bud development
566 in potato, but due to internal control by StSP6A, flower bud development is promoted under
567 long-day conditions.
568
569 *Interaction between two modes of reproduction in potato*
570 A likely mode of action for StSP6A to impair flower bud development, could be through
571 control of assimilates. Although removing the tuber-sink did not improve flower bud
572 development, it cannot be claimed assimilates do not play a role, as alternative tuber structures
573 that acted as sinks were still formed unless *StSP6A* was silenced. StSP6A may have a role in
574 directing assimilates towards tuberization, which consequentially could be detrimental for
575 flower development, especially if tuberization takes place while flower buds are still

576 developing. How this direction of assimilates takes place remains to be elucidated. Whether
577 flowering is actually repressed by StSP6A may be genotype specific and depend on the timing
578 of both tuberization and flowering. The European Cultivated Potato Database
579 (https://www.europotato.org) shows a huge variation in flowering success between varieties
580 and it has been suggested that potato berry and seed development is impeded by earliness of
581 tuberization (Pallais, 1987). Similar findings were seen in the CE3027 and CE3130 control
582 grafts, where the early tuberizing CE3130 grafts flowered less profusely than the later
583 tuberizing CE3027 grafts (Fig. 3B and Supplementary Table S2). It would be interesting to
584 correlate the tuberization time and *StSP6A* expression to the flowering time and flower
585 developmental success in a large number of genotypes.
586 While two reproduction modes may inhibit each other in the same species, interspecies
587 interaction between reproduction modes may be beneficial for both processes, as was seen in
588 *S. etuberosum* scions grafted on CE3027 and CE3130 rootstocks. The flowering in *S.*
589 *etuberosum* scions was improved compared to control grafts with *S. etuberosum* rootstocks.
590 StSP6A may not function as an inhibiter in *S. etuberosum* because flowering and tuberization
591 are not competing processes in this species. Consequently, StSP6A may substitute FT in *S.*
592 *etuberosum* and improve flowering, while StSP6A inhibits flowering in potato.
593
594 *Conclusion*
595 Our results show that flower bud development in potato is impaired by the tuberization signal
596 StSP6A, and not by the tuber-sink itself. These results suggest there is an internal mechanism
597 in potato plants where one mode of reproduction can affect the other.
598
599
600 **SUPPLEMENTARY DATA**
601
602 **Table S1.** Overview of the five experiments testing how tuberization affects potato flower bud
603 development.
604 **Table S2.** Flower bud development and tuberization in grafts between CE3130 and *S.*
605 *etuberosum*.
606
607 **Figure S1.** *StNAC* expression (Ct = cycle threshold) in wild-type *S. andigena, StSP6A RNAi*
608 #1, and *StSP6A RNAi* #13.
609 **Figure S2.** *StSP6A* expression and tuberization time in wild-type *S. andigena, StSP6A RNAi*

610 #1, and *StSP6A RNAi* #13.

611

612

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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).**

| Scion | Rootstock | *n* | Anthesis * | Max. open flowers/plant | Tuber dry weight (g/plant) |
|---|---|---|---|---|---|
| *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.

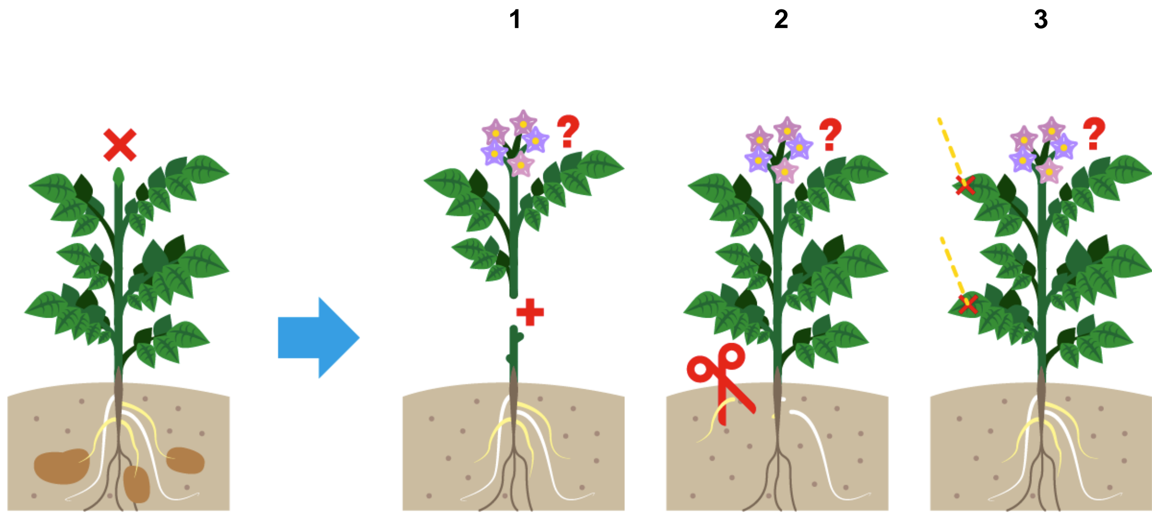


**Figure 1.**

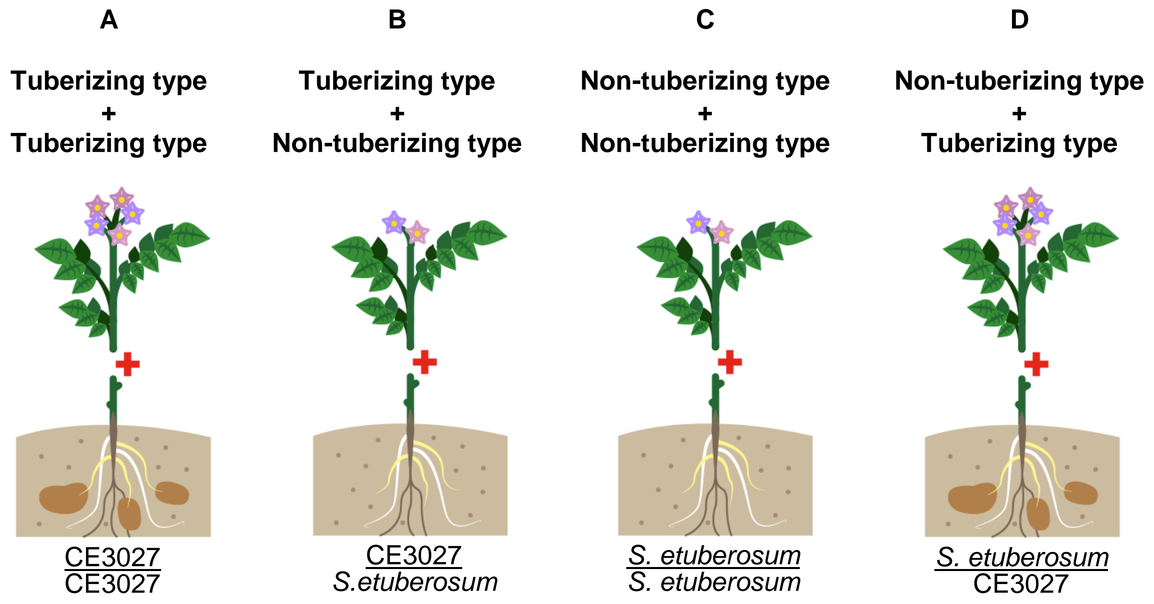


**Figure 2.**

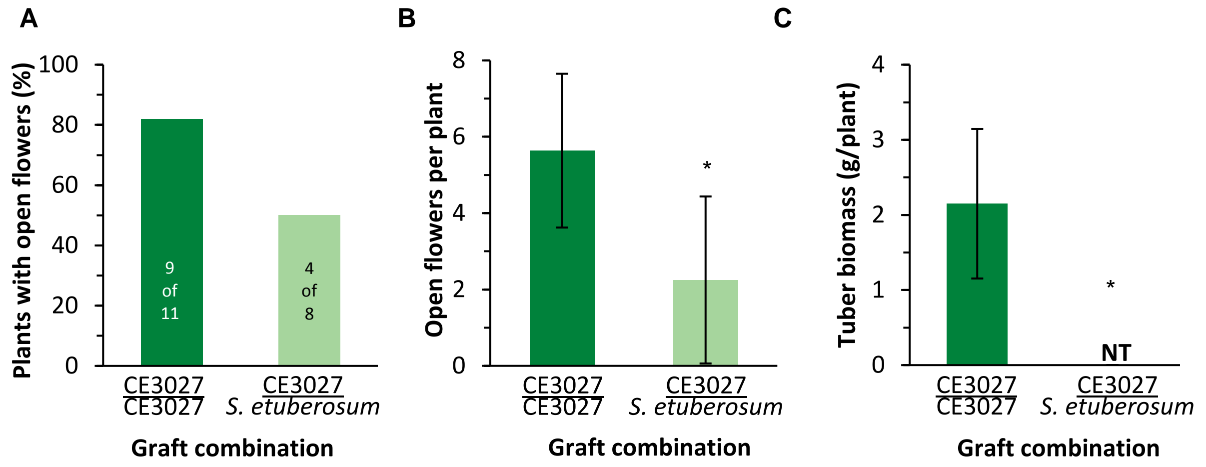


**Figure 3.**

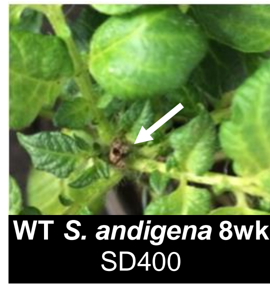

**Figure 4.**

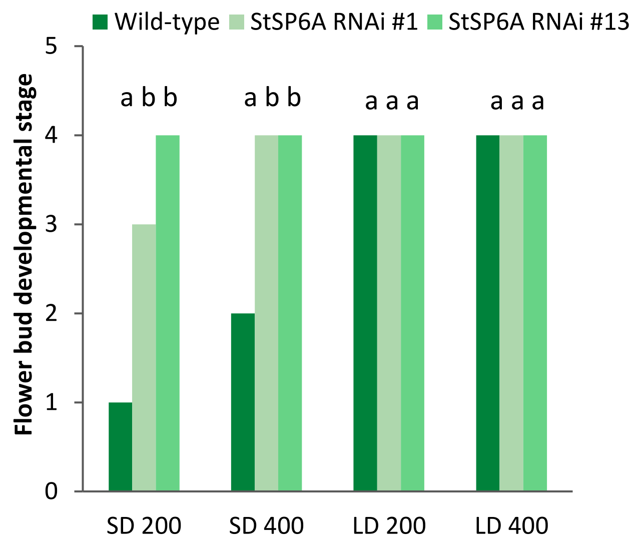

**Figure 5.**

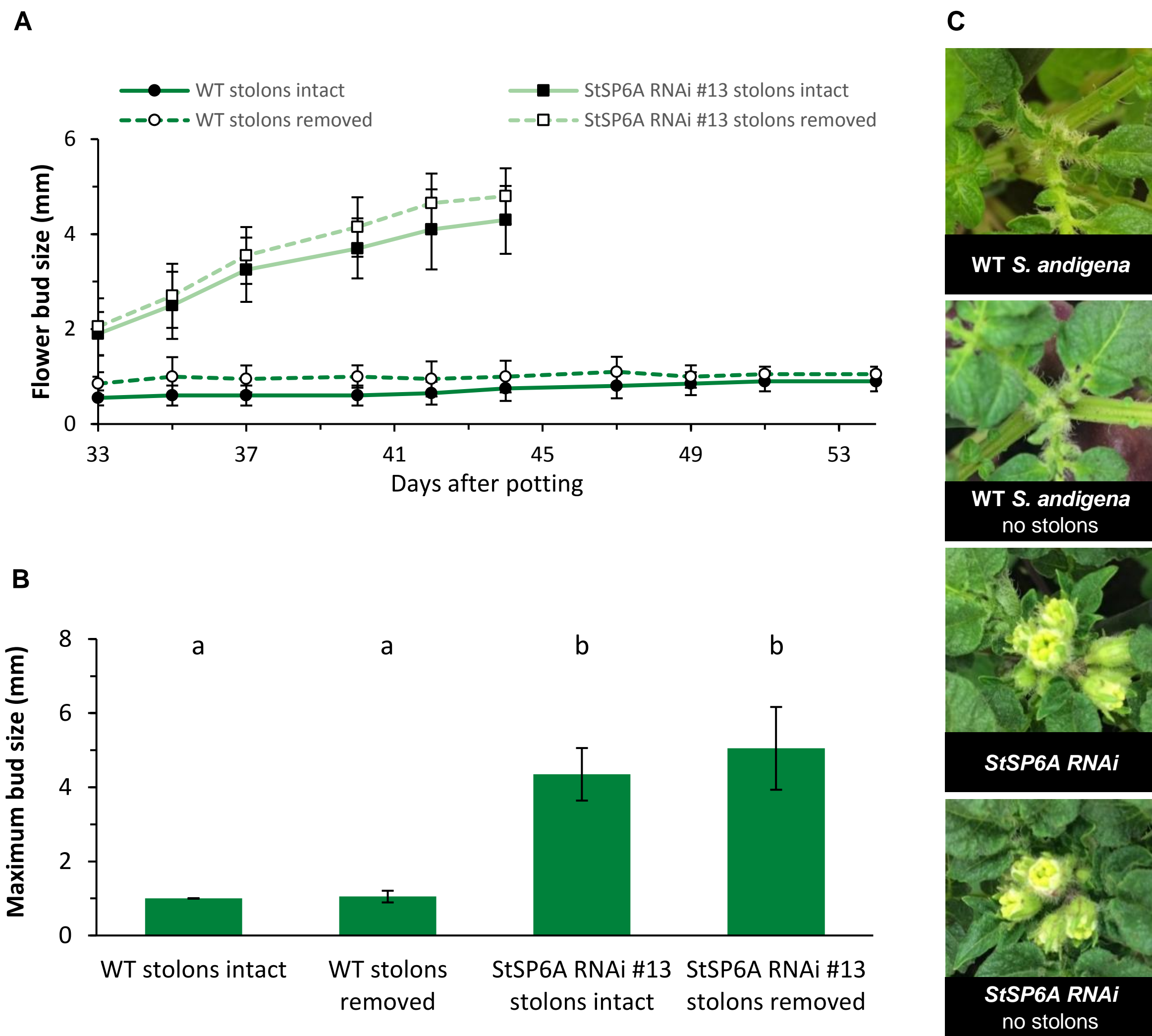


**Figure 6.**

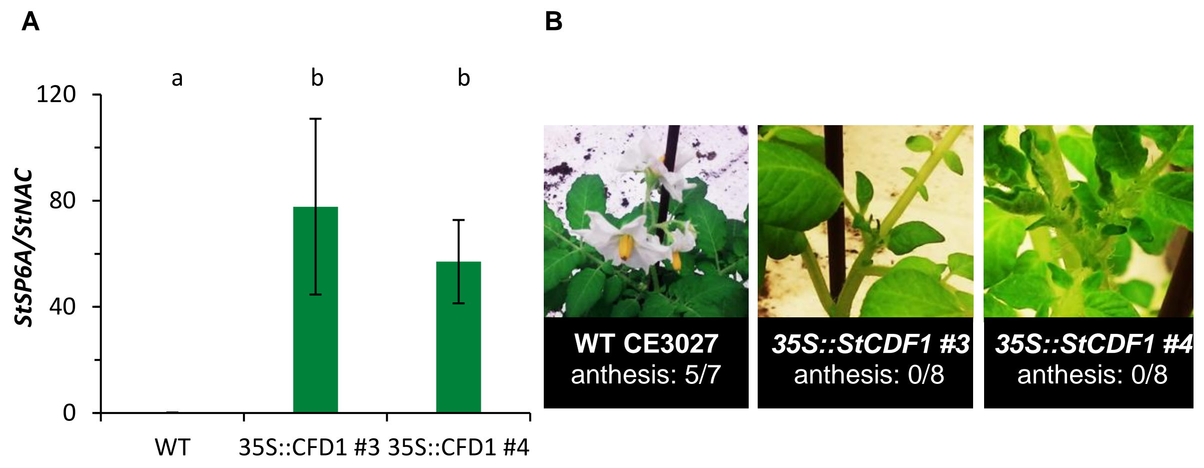


**Figure 7.**

A

B

C

D

**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<br>*S. etuberosum*<br>*S. palustre* | 8, 22/18**, not measured (winter-spring 2016), not measured, 14, greenhouse |
| 1b | **Removing tuber-sink**: Grafting | 10* | CE3027<br>CE3130<br>*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<br>5<br>5 | *S. andigena* WT<br>*StSP6A RNAi#1*<br>*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*<br>*StSP6A RNAi#13* | 8, 22/18, 400, 70%, 8, climate chamber |
| 5 | **Increasing the tuber signal:** Overexpressing *StCDF1* | 10<br>8<br>8 | CE3027<br>*35S::StCDF1 #3*<br>*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 ($\alpha = 0.05$)

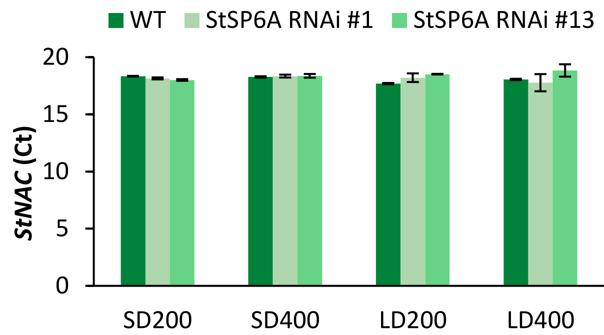


**Figure S1. *StNAC* expression (Ct = cycle threshold) in wild-type *S. andigena, StSP6A RNAi* #1, and *StSP6A RNAi* #13**. The reference gene *StNAC* is similarly expressed in all tested light treatments and genotypes. Plants were grown in short days (SD) of 200 and 400 µmol·m$^{-2}$·s$^{-1}$ and long 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.

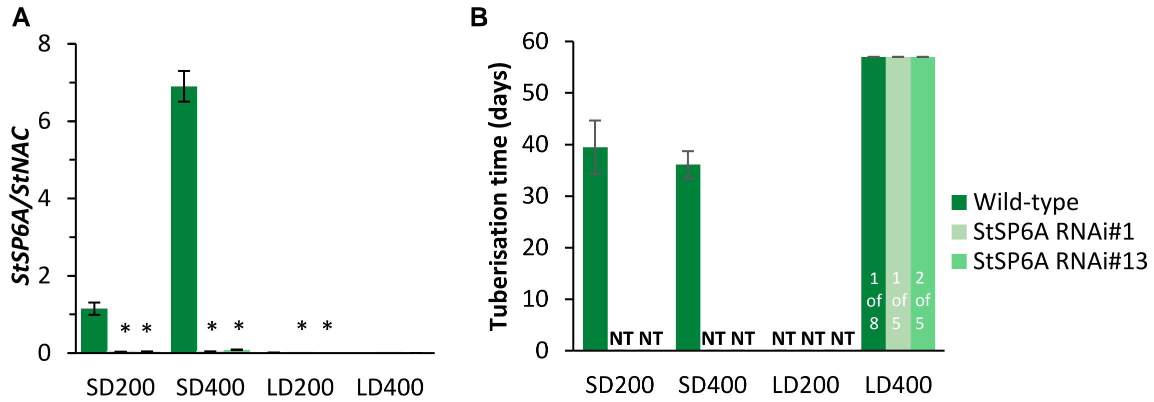


**Figure S2. *StSP6A* expression and tuberization time in wild-type *S. andigena, StSP6A RNAi* #1, and *StSP6A RNAi* #13**. Plants were grown in short days (SD) of 200 and 400 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and long days (LD) of 200 and 400 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. (A) *StSP6A* is expressed relative to the reference gene *StNAC*. Expression is from plants five weeks after transplanting. The error bars show the standard 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).