The sucrose transporter StSUT4 from Solanum tuberosum affects flowering, tuberization and shade avoidance response

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running title: StSUT4 involved in the perception of light quality
Summary

Sucrose transporters belong to a large gene family. The physiological role of SUT1 proteins has been intensively investigated in higher plants, while that of SUT4 proteins is so far unknown. All three known sucrose transporters from potato SUT1, SUT2 and SUT4 are co-localized and their RNA levels not only follow a diurnal rhythm, but oscillate in constant light. Here, we examined the physiological effects of transgenic potato plants on RNAi-inactivated StSUT4-expression. The phenotype of StSUT4-RNAi plants includes early flowering, higher tuber production and a reduced sensitivity towards light enriched in far-red wavelength (i.e. in canopy shade). Inhibition of StSUT4 led to tuber production of the strict photoperiodic variety Solanum tuberosum subsp. andigena even under non-inductive LD conditions. Accumulation of soluble sugars and sucrose efflux from leaves of the transgenic plants are modified in StSUT4-RNAi plants leading to modified sucrose levels in sink organs. StSUT4 expression of wild-type plants is induced by gibberellins and ethephon, and external supply of GA$_3$ leads to even more pronounced differences between WT and StSUT4-RNAi plants regarding tuber yield and internode elongation, indicating a reciprocal regulation of StSUT4 and gibberellins.

Keywords: flower induction, tuberization, photoperiod, shade avoidance response, GA signaling, sucrose signaling

Abbreviations: SD short day, LD long day, GA gibberellic acid, phy phytochrome, WT wild type
Introduction

Phylogenetic analysis of the sucrose transporter gene family shows redundancies in the SUT1 clade representing transporters involved in phloem loading and long distance transport of sucrose (Riesmeier et al., 1993; Kühn, 2003). The well characterized members of the SUT1 family are highly expressed and essential in phloem loading. In contrast, the SUT2 and SUT4 families are represented with only one member each per species and expressed at a very low level suggesting a function different from SUT1 (Kühn, 2003). For example, LeSUT2 plays an important role in pollen tube growth and pollen germination thereby affecting fruit yield in tomato plants (Hackel et al., 2006).

The function of the SUT4 protein remains to be elucidated. The activity of SUT4 proteins has been shown by sucrose uptake experiments and yeast complementation with AtSUT4 from Arabidopsis, StSUT4 from potato (Weise et al., 2000; Weschke et al., 2000) and the orthologous HvSUT2 from barley (Weise et al., 2000; Weschke et al., 2000). StSUT4 and LeSUT4 have been immunolocalized to the plasma membrane of phloem sieve elements in potato and tomato respectively (Weise et al., 2000; Weschke et al., 2000) as it was previously demonstrated for StSUT1 and LeSUT1 (Kühn et al., 1997). The yeast two hybrid split ubiquitin system revealed interaction of the LeSUT4 protein with the co-localised LeSUT1 in yeast (Reinders et al., 2002). In comparison to StSUT1, the expression level of StSUT4 is very low (Weise et al., 2000). Therefore, the expression pattern and the function of SUT4 genes have not been analyzed in detail.

The aim of our work is the elucidation of the under-characterized sucrose transporter StSUT4 by help of transgenic plants. We addressed the putative role of StSUT4 in the regulation of flower induction and tuberization in potato plants. Solanum tuberosum is a short day plant regarding tuberization and a long day plant regarding flowering (Schittenhelm et al., 2004). Flower induction is a very well investigated developmental process in higher plants and is mediated by different signal transduction pathways including the photoperiod-dependent phytochrome signal transduction pathway, the sucrose pathway, and the gibberellic acid pathway (reviewed by Searle and Coupland, 2004; Thomas, 2006). It is still unclear, whether these flower-inducing pathways act independently or synergistically via a common signaling pathway.

Photoperiodic regulation is not only important to determine the flowering time in many plants, but promotes tuberization in potato as well. Flowering tobacco shoots grafted onto potato stocks promote tuberization, indicating that the floral and tuber-inducing signals might be similar. Thus, common regulatory pathways were assumed to be...
involved in both flowering and tuberization responses (Rodriguez-Falcon et al., 2006). Overexpression of the Arabidopsis flowering-time gene CONSTANS induced tuberization in potato plants (Martinez-Garcia et al., 2002) and it is suggested that the function of the potato orthologs of CONSTANS and FLOWERING LOCUS T (FT) is conserved for tuberization control.

Inhibition of the StSUT4 expression in transgenic potato plants by RNA interference (RNAi) leads to early flowering and increased tuber yield. The interrelation between the observed phenomena with photoperiodic control was tested by using the strictly photoperiodic potato variety Solanum tuberosum L. ssp. andigena instead of S. tuberosum ssp. tuberosum. Graft experiments showed that the flower and tuber inducing stimulus is graft-transmissible and requires the presence of source leaves, arguing for a phloem-mobile leaf-derived signal. In addition, StSUT4-RNAi plants do not alter the elongation of internodes, leaf angle, flowering or apical dominance in response to shading by neighbouring plants or in response to far red light enrichment. They do not display what is summarized by the shade avoidance syndrome (SAS), suggesting that photoreceptor signaling is deregulated. It will be discussed whether there is convergence of the signal transduction mechanisms triggering flowering, tuberization and shade avoidance response.
Results

**StSUT4 is a plasma membrane protein**

Whereas *StSUT1* is mainly expressed in exporting source leaves (Riesmeier et al., 1993), the expression pattern of *StSUT4* is highest in sink organs. The StSUT4 protein has previously been localized in phloem sieve elements (Weise et al., 2000) and our expression studies indicate that *StSUT4* transcripts accumulate during flower and tuber development (Fig. 1A).

StSUT4 is functional in sucrose uptake in yeast cells (Weise et al., 2000). Thus, a plasma membrane specific localization is a prerequisite for this sucrose uptake. Two-phase partitioning of plant microsomal membranes separating the plasma membrane and endosomal membrane fraction revealed preferential localization of StSUT4 in the plasma membrane fraction (Fig. 1B) as shown by the use of a StSUT4-specific peptide antibody (Weise et al., 2000). In the microsomal and the endosomal fraction a smaller band of 38 kDa is recognized by the StSUT4-specific antibody which might represent a degradation product of the StSUT4 protein. Western Blot analysis was confirmed by localization studies with a StSUT4-GFP fusion protein in infiltrated tobacco and potato leaves. The protein was localized at the plasma membrane (Fig. 1C), and in addition, in a peri-nuclear ring (Fig. 1D-G). A similar phenomenon is observed when *LeSUT4* from *Lycopersicon esculentum* fused to GFP is expressed in yeast cells (Fig. 1H). Expression of GFP fusion constructs in yeast and plant cells are interpreted as dual targeting of SUT4 to both the plasma membrane and endomembranes surrounding the nucleus.

**Sucrose transporters show oscillation in constant light**

The SUT1 transcript levels from tomato show a diurnal oscillation with the highest expression at the end of the light period (Kühn et al., 1997). We performed real time PCR analysis of all known sucrose transporter genes in WT potato plants. Fig. 2 represents oscillating sucrose transporter transcript levels in potato leaves under constant light conditions. *StSUT1* and *StSUT4* show a similar expression pattern with maximum transcript levels in the middle of the light period, whereas *StSUT2* mRNA peaks at the beginning of the light period (Fig.2B). RNA levels continue oscillation within 64h of continuous illumination arguing for circadian regulation of the sucrose transporter gene expression. Moreover, elements for circadian regulation of transcription were found in the promoter sequences of sucrose transporters when analyzed with the Web Signal Scan Program ([http://www.dna.affrc.go.jp/sigscan/signal1.pl](http://www.dna.affrc.go.jp/sigscan/signal1.pl)). According to Harmer and Kay an imperfect evening element with
the consensus AAAATATCT is present in the LeSUT1 promoter sequence (Harmer and Kay, 2005).

Inhibition of SUT4 leads to early flowering and tuberization

As much as nine independent transformant lines have been identified with efficiently down-regulated expression of the \textit{StSUT4} gene (Supplement 1). PCR analysis confirmed the presence of the \textit{StSUT4-RNAi} construct and real time PCR revealed reduction of \textit{StSUT4} expression in the tissue of highest endogenous \textit{StSUT4} RNA content. The \textit{StSUT4} expression level is reduced up to 67\% in flowers compared to WT flowers. \textit{StSUT4} inactivation is specific and \textit{StSUT1} expression was not significantly altered in \textit{StSUT4-RNAi} plants (Supplement 2). Seven transformant lines with significant reduction of \textit{StSUT4} expression in flowers were selected for further analysis. After four weeks of growth under LD conditions, potato plants with reduced \textit{StSUT4} expression started to flower at least 6 days before WT flowering (Fig. 3A, Fig. 4A). \textit{StSUT4-RNAi} plants had significantly fewer leaves at flowering time than WT plants (Fig. 4A). Neither \textit{S. tuberosum} Désirée WT plants, nor \textit{StSUT4-RNAi} plants were able to flower under SD conditions. The selected transformants showed a reduced internode elongation regardless of the day length or the light quality (Fig. 3B, Fig. 8B, Supplement 3). Moreover, they tuberized earlier than control plants and showed significantly increased tuber yields (with p<0.05) when grown under LD conditions (Fig 4B, Table 1) and only slightly increased yield when grown under SD conditions compared to WT plants (data not shown).

Whereas \textit{Solanum tuberosum} subsp. tuberosum is able to tuberize under LD conditions, \textit{Solanum tuberosum} subsp. andigena requires an obligatory SD period for tuberization and does not tuberize under LD conditions (Jackson et al., 1998). We tested whether the effect of \textit{StSUT4} on tuberization depends on the day length. Transformation of the strictly photoperiodic potato subsp. andigena was performed with the same \textit{StSUT4-RNAi}-construct as used for transformation of \textit{S. tuberosum} Désirée. Six independent transformant lines with reduced \textit{StSUT4} expression were selected (Supplement 1, up to 50\% reduction of the \textit{StSUT4} expression level in source leaves of the transgenic potato plants). All six transformant lines (i) were able to produce tubers in at least two out of three independent experiments when grown in the greenhouse under non-inductive LD conditions (Fig. 3 D, E), (ii) had shorter internodes (Fig. 3C) and (iii) showed early flowering compared to andigena WT plants (Fig. 3F). The ability to produce tubers even under LD conditions is correlated with a reduction of the \textit{StSUT4} expression in these plants (Supplement 1). Thus, nine independent transgenic lines of \textit{S. tuberosum} ssp. tuberosum and in 6 independent
transgenic lines of the photoperiodic *S. tuberosum* ssp. andigena show similar effects on flowering and tuberization upon inhibition of StSUT4 expression.

**SUT4 mediated flower and tuber induction is graft-transmissible**

In order to analyze whether the flower and tuber-inducing signal is graft-transmissible, reciprocal grafts were performed with transgenic *StSUT4*-RNAi and wild-type potato plants (subsp. tuberosum and andigena) after the plants had developed 4-5 leaves, and with or without removal of the source leaves of the corresponding graft rootstock. Flowering time was recorded and tubers of grafted plants were harvested 3 months after transfer of the plants into the greenhouse.

Wild type potato plants from both subspecies, which were grafted with *StSUT4*-RNAi plants that included their leaves, showed a similar phenotype as transgenic *StSUT4*-RNAi plants: they displayed early flowering (Fig. 5A) and produced higher amounts of tubers as compared to grafts with control plants (Fig. 5C). Therefore, it is suggested, that a phloem-mobile information molecule is involved in triggering flowering time and tuberization in a SUT4-dependent manner. Both, early flowering as well as higher tuber yield strongly depend on the presence of source leaves of the root stock regardless of its genotype. It can not be excluded that the reduced total leaf area available for assimilation has an impact on the tuber yield.

**SUT4 inhibition induces increased sucrose efflux and changes in sugar accumulation**

A detailed analysis of the content of soluble sugars at different time points over the day revealed significant differences between WT and RNAi plants depending on the time of day (Fig. 6A-C). While soluble sugars are present in lower concentration in the source leaves of transgenic plants than in those of WT plants at the beginning and in the middle of the light period, they accumulate to much higher amounts at the end of the light period. This increase in the content of soluble sugars in *StSUT4*-RNAi plants is accompanied by an increase in sucrose efflux. The sucrose export from petioles of the transgenic plants as measured by exudation in the presence of EDTA was twice as much as in WT plants at the end of the light period (Fig. 6D).

As expected due to the strong increase of sucrose efflux rates at the end of the light period, the sucrose content in sink organs was in consequence affected as well. The sucrose as well as the starch content is significantly increased in *in vitro* induced microtubers of *StSUT4*-RNAi plants compared to WT tubers (Fig. 6E) The sucrose content in the shoot apical meristems was measured at different developmental
stages (Fig. 6F). Whereas the content of glucose and fructose was not significantly changed between WT and transgenic plants, the level of sucrose differs conspicuously. As already described in Arabidopsis (Eriksson et al., 2006), a sucrose peak can be observed in the shoot apical meristem shortly before flower onset, when WT undergoes transition from the vegetative to the generative phase. In contrast, sucrose levels in StSUT4-RNAi apical meristems show peak levels much earlier. This correlates with early flower induction in the transgenic plants. Floral buds are detectable when the transgenics developed 5-6 mature leaves, whereas WT plants had more than 10 mature leaves when first buds are visible (Fig. 6F). Thus, modified sucrose efflux from leaves is accompanied by changes of sucrose levels in terminal sinks.

**StSUT4-RNAi plants do not show shade avoidance**

The transgenic plants have shorter stems due to reduced internode elongation, show early flowering and higher tuber yield. Tuberization in potato is negatively controlled by gibberellins and phytochrome B (Jackson and Prat, 1996). Phytochrome B is involved in the photoperiodic control of tuberization in S. tuberosum subsp. andigena (Jackson and Prat, 1996). Plants with reduced levels of phytochrome B tuberize in LD as well as in SD conditions, whereas WT plants will only tuberize under SD conditions (Jackson et al., 1996). StSUT4-RNAi plants are comparable to phytochrome B antisense plants regarding tuberization and flowering (Jackson et al., 1998). They are also comparable to transgenic plants with reduced biosynthesis of gibberellins, as these also show shorter stems, reduced internode elongation and tuberized earlier (Carrera et al., 2000). The phenotype of StSUT4-RNAi plants would therefore be consistent with a reduced amount of phytochrome B, or a reduced biosynthetic capacity for gibberellic acids. The transcript levels of phyA and phyB in StSUT4-RNAi plants were determined by quantitative real time-PCR. Neither phyB, nor phyA expression was significantly affected in StSUT4-RNAi plants (not shown). At least for tobacco plants it was shown that ethylene and gibberellins interact in the phytochrome-mediated shade avoidance syndrome (Pierik et al., 2004). In order to test if StSUT4 is under phytohormonal control, WT potato plants were treated with GA₃ and paclobutrazol, with ethephon, a soluble ethylene precursor, and with silver nitrate, an efficient inhibitor of the ethylene receptor. Here, we observed that the StSUT4 expression in WT potato plants was induced by GA₃ treatment at the end of the light period, and a significant increase of the SUT4 expression was also observed in ethephon treated WT leaves at all time points (Fig. 7A).
The transcript levels of both, ethylene and GA biosynthetic key enzymes were determined by quantitative real time PCR. Quantification of the transcript level of the GA biosynthetic enzyme GA20ox1 as well as ACC oxidase StACO3 showed significant changes in StSUT4-RNAi plants compared to potato WT plants (Fig. 7B+C). The level of GA20ox1 is significantly decreased in StSUT4-RNAi plants at dawn, which might explain the increase in tuber yield and the reduction of internode elongation (Fig. 7B).

StSUT4 expression is not only increased by gibberellins, but also by ethephon treatment, a precursor of ethylene (Fig. 7A). Quantification of the transcript level of the ethylene biosynthetic enzyme ACC oxidase StACO3 was found to be significantly decreased in StSUT4-RNAi plants at any time of day (Fig. 7C). Ethylene as well as GA biosynthesis might therefore be affected in StSUT4-RNAi plants.

Stem elongation and early flowering belong to the shade avoidance syndrome. The shade avoidance response is phyB-mediated and antagonized by phyA (Vandenbussche et al., 2005). In order to test the ability of the transgenic plants to display the phyB-mediated shade avoidance response, the plants were grown at high density in order to shade the source leaves by the canopy of neighboring plants. Although the phyB transcript level is not decreased in StSUT4-RNAi plants, the typical shade avoidance response cannot be observed. Stem elongation under decreased red:far-red light ratio by canopy shade increased to a much lower extent in StSUT4-RNAi plants than in WT plants (Fig. 8).

The experiment was repeated under artificial shade conditions in the phytochamber, where plants were grown under white light or under white light with an additional source of far red light (>730 nm). After three weeks of growth under these artificial shade conditions, internode elongation of StSUT4-RNAi plants was not significantly increased if compared to identical plants grown under white light, whereas WT plants showed significantly increased internode elongation and stem length as expected (Figure 8C,D). Thus, StSUT4-RNAi plants behave similar under canopy shade as under far red light enrichment.

**GA signaling is affected in StSUT4-RNAi plants**

Since StSUT4-RNAi plants behave similar to GA20oxidase1 antisense potato plants regarding internode elongation, stem length and tuberization (Carrera et al., 2000), we aimed to rescue the WT phenotype of our RNAi plants by application of external GA$_3$. GA$_3$ was sprayed directly on source leaves over a 2 weeks period in a concentration of 20 µM supplemented with Triton-X 100, whereas control plants were treated with water with Triton-X 100. The exogenous GA$_3$ supplied was not able to
rescue the WT phenotype regarding stem elongation, tuber yield or flowering (Fig. 9). The differences between WT and transgenic plants were even more pronounced than in water-treated plants.

Regarding tuber yield, not only the tuber size was increased in StSUT4-RNAi plants, but also the tuber number (Fig. 9B) indicating that not only the starch accumulation and tuber development is disturbed but also the induction of tuberization. However, paclobutrazol, a specific inhibitor of GA biosynthesis, was able to mimic the StSUT4-RNAi phenotype in WT plants. Paclobutrazol treatment resulted in the same internode length in wild-type and StSUT4-RNAi plants (Fig. 9C) indicating that GA-induced response is already impaired in StSUT4-RNAi plants.
Discussion

Localization of SUT4-GFP in plant cells

Members of the SUT4 subfamily, namely AtSUT4 from Arabidopsis, StSUT4 from potato and HvSUT2, the SUT4 orthologue in barley, are able to contribute to the 14C-sucrose uptake into yeast cells at the plasma membrane (Weise et al., 2000; Weschke et al., 2000). StSUT4 and LeSUT4 have been immunolocalized at the plasma membrane of sieve elements, and AtSUT4 promoter::GUS studies revealed highest AtSUT4 expression in minor veins (Weise et al., 2000). Our localization experiments using GFP constructs are consistent with these previous results (Fig. 1).

The StSUT4-GFP fusion protein was found at the plasma membrane of infiltrated tobacco and potato leaves, and a LeSUT4-GFP fusion protein was localized at the plasma membrane of yeast cells (Fig. 1). Nevertheless, members of the SUT4 family have been identified by proteomic approaches either in the chloroplastic fraction in Arabidopsis (Rolland et al., 2003) or in the vacuolar fraction in Arabidopsis and barley (Endler et al., 2006). SUT4-mediated sucrose uptake in yeast cells was explained by mistargeting of the proteins in yeast, where it is detectable at the plasma membrane as well as in internal membrane structures. Our localization data with StSUT4-GFP in plant cells revealed a very similar distribution of the fusion protein both at the plasma membrane and in internal membranes surrounding the nucleus (Fig. 1). We suggest that SUT4 from Solanaceae is located in both, the plasma membrane and the endomembrane system and undergoes dual targeting. It cannot be excluded, that the localization of the SUT4 protein underlies dynamic changes leading to localization in different compartments. Species-specific differences might be the reason for the different localization of AtSUT4 and StSUT4.

StSUT4 affects sucrose efflux from leaves and sucrose levels in sinks

SUT1 is the most important sucrose transporter for the efflux of sucrose from mature leaves, since it is highly expressed in source leaves. SUT2 and SUT4 expression is more prominent in sink tissues (Fig. 1A). Our transcript analysis of StSUT4 confirms it low expression and revealed a significant reduction of StSUT4 in flowers of StSUT4-RNAi plants (Supplement 1). StSUT1 mRNA levels are unaffected in StSUT4-RNAi plants (Supplement 2). Thus, a transcriptional control of SUT1 via SUT4 is unlikely. Nevertheless, sucrose efflux from leaves is significantly increased at the end of the light period in StSUT4-RNAi plants and sucrose content is increased in in vitro grown tubers and at earlier stages in the shoot apical meristem of StSUT4-RNAi plants (Fig. 6). Changes in source to sink allocation in the transgenic plants might be one reason for the early onset of flowering and tuberization.
Overexpression of a SUT1 gene in transgenic tobacco plants lead to a similar early flowering phenotype as observed for StSUT4 inhibition in potato plants (Riesmeier and Frommer, 1994). StSUT4 might play a role as inhibitor of StSUT1, and increased sucrose efflux from leaves of StSUT4-RNAi plants is then explained by the missing StSUT4-mediated StSUT1 inhibition.

It is known from yeast two hybrid studies, that the LeSUT4 protein is able to interact with LeSUT1 protein in yeast (Reinders et al., 2002). Therefore, a post-translational regulation by heterodimerization of the StSUT1, the main phloem loader in potato and the StSUT4 protein cannot be excluded.

**SUT4 is involved in shade avoidance**

The shade avoidance syndrome (SAS) is a very complex reaction of plants towards canopy shade of neighbouring plants involving photoperiodic control and the interaction of phytochrome and blue light receptors. The SAS is not only triggered by the red/far red light ratio via phytochromes, but also by a reduction of blue light under canopy shade (Pierik et al., 2004). Circadian gating also plays an important role in shade avoidance response (Vandenbussche et al., 2005).

PhyB is known to inhibit flowering in LD plants like *Arabidopsis*, since phyB mutants show early flowering (Guo et al., 1998). Antisense potato plants, in which the phyB expression is down-regulated, tuberize earlier under LD conditions (Jackson et al., 1996; Jackson et al., 1998) as found for StSUT4-RNAi plants. It was shown that phyB at least in LD plants affects flowering negatively via inhibition of CONSTANS and the FLOWERING LOCUS T (Cerdan and Chory, 2003; Endo et al., 2005). Thus, phyB inhibits tuberization and flowering in LD conditions and is relevant for the induction of a shade avoidance response under a far-red enriched light regime. StSUT4-RNAi plants flower and tuberize earlier and do not show shade avoidance response suggesting that the mediation of the photoreceptor signal transduction correlates with sufficient SUT4 levels. As StSUT4-RNAi plants have a lower phyB-mediated shade avoidance response, the phytochrome-mediated inhibition of flowering parallels with StSUT4 expression in potato plants. Thus, in StSUT4-RNAi plants the phyB transcript level is unaffected, but StSUT4 seems to be required to transfer the phyB-emitted signal further downstream.

StSUT4 might act downstream of photoreceptors detecting the light quality in source leaves, and upstream of ethylene and gibberellins (as summarized in Fig. 10). So far, it is known that both, photoreceptors, sugar and phytohormones such as ethylene...
and gibberellins are involved in shade avoidance (Pierik et al., 2004; Kozuka et al., 2005), but it is still unclear, how these two signaling pathways are interconnected to each other.

**SUT4 is involved in GA signaling**

PhyB action negatively affects flowering in LD plants and inhibits tuberization in potato plants (Jackson and Prat, 1996; Endo et al., 2005). Graft experiments between phyB antisense and WT potato plants revealed that a graft-transmissible inhibitor of tuberization is responsible for inhibition of potato tuber induction under non-inductive LD conditions (Jackson et al., 1998). It is also known that phytochromes act by transferring a leaf-derived signal towards the shoot apical meristem to induce flowering (Valverde et al., 2004).

In tobacco plants, the phytochrome-mediated shade avoidance response involves ethylene action by modulating GA action (Pierik et al., 2004). It is also known, that phyB and light regulate the GA₃ biosynthesis (Reed et al., 1996).

The phenotype of StSUT4-RNAi plants including a decreased length of internodes and early tuberization leading to higher tuber yields was exactly described for plants with reduced expression of GA20ox1 (Carrera et al., 2000). In addition, the StSUT4-RNAi plants show early flowering. The overall phenotype of StSUT4-RNAi plants includes also reduced level of GA20ox1 at the end of the day and is in accordance with reduced biosynthesis of gibberellins. Thus, a reciprocal regulation of StSUT4 and gibberellins is assumed.

A feedback control of gibberellic acid biosynthetic enzymes by GA₃ and diurnal oscillation in potato under SD conditions has already been described (Carrera et al., 1999). External application of gibberellins to StSUT4-RNAi leaves was not able to rescue the WT phenotype. The reason might be the negative feedback regulation of GA20oxidase1 by external GA₃ application (Carrera et al., 1999) leading to even more severe effects in StSUT4-RNAi plants where GA biosynthesis is already down-regulated. Involvement of StSUT4 in GA signaling is strongly supported by the fact that inhibition of GA biosynthesis by paclobutrazol affects stem elongation of WT potato plants, mimicking the phenotype of StSUT4 inhibition and leading to the same internode length in both sets of plants.

**Sucrose as signaling molecule**

Strong expression of StSUT4 in flowers and tubers argues for an important role of this membrane protein in sink organs. Nevertheless, the observed effects regarding photoperiodically regulated developmental processes in the StSUT4-RNAi plants like
early flowering and tuberization under LD conditions are graft-transmissible and depend on the presence or absence of source leaves indicating an important role of SUT4 not only in sink tissues, but also in source leaves where photoperception occurs. Therefore, a long distance component is needed to transmit the information from photoreceptors in leaves to GA biosynthesis in tubers and flower induction in the shoot apical meristem. Several phloem mobile signaling molecules are discussed and sucrose itself might play a role as a phloem mobile signaling molecule (Smeekens, 2000).

It is also discussed that assimilates act as a part of a complex flowering signal (Bernier and Perilleux, 2005), since photosynthesis and photoperiodism were shown to interact in flower induction (Friend, 1984). The Arabidopsis flowering time in non-inductive SD conditions is determined by sharp increases of GA4 and sucrose in the apical meristem shortly before flower initiation (Eriksson et al., 2006) and both, GAs and sucrose, are discussed to be part of the florigenic signal. Alternatively, the phloem mobility of FT might be dependent upon a sufficient mass flow of assimilates (Thomas, 2006). It is known, that tuberization in potato depends on StCOL3 and StFT interplay (Rodriguez-Falcon et al., 2006) and we showed that the accumulation of sucrose transporter mRNAs follow circadian oscillation. Thus, it can not be excluded, that StSUT4 affects the photoperiodic pathway via the level of the florigenic and tuberigenic proteins StCOL3 and StFT (as postulated in the model in Fig. 10).

We were able to show, that peak sucrose levels are detectable earlier in the apical meristem of StSUT4-RNAi plants which is a strong argument for the sucrose molecule to be necessary to build up a flower inducing component in potato plants. Temporal and spatial fine-tuning of sucrose concentrations as well as GA levels seems to be extremely important to integrate flower and tuber inducing mechanisms. Therefore, we conclusively suggest that StSUT4 seems to play an important role in the interconnection of carbon availability with flower inducing mechanisms thereby linking light quality with light quantity effects on flowering and tuberization.
Experimental procedures

Recombinant DNA. Isolation of the StSUT4 cDNA was described previously (Weise et al., 2000). For GFP fusion, the multiple cloning site of the vector pCF203 was modified and additional restriction sites were inserted via synthetic oligo linker (SacI, KpnI, SpeI, XbaI, XhoI, BamHI cloned into the SacI and BamHI restrictions sites of pCF203). pCF203 carries GFP under control of the cauliflower mosaic virus 35S promoter. StSUT4 cDNA was amplified with primers with restriction sites for KpnI and EcoRV (fw TAT GGT ACC ATG CCG GAG ATA TAG AAA GG, rev GAT GAA TAT CTG TGC AAA GAT CTT GGG TTT C) and cloned in the modified pCF203 linearized with BamHI, blunted and redigested with KpnI. For LeSUT4 fusion to GFP the LeSUT4 was amplified from cDNA using proof-reading DNA-polymerase and cloned via PstI and NotI restriction sites together with the NotI-EcoRI fragment of GFP into the yeast expression vector 112A1NE (Riesmeier et al., 1992) linearized with PstI and EcoRI.

The RNAi construct was cloned into the pRT 100 derivative (Töpfer et al., 1987), pRT-RNAi (Hirner et al., 2006) kindly provided by Axel Hirner and transferred into pJH212. For construction of the RNAi construct, a 989 bp fragment of the StSUT4 cDNA was amplified with primer fw TAT GGT ACC ATG CCG GAG ATA TAG AAA GG and rev GAGA CTC GAG TGC AAA GAT CTT GGG TTT CTG, digested with XhoI and Smal, and cloned into the SalI and Smal sites of pRT-RNAi. A second StSUT4 fragment (XhoI-Smal digested) was inserted via the XhoI and Eci136I sites into pRT-RNAi. A 3,5 kb PstI fragment containing both StSUT4 fragments was afterwards transferred into the PstI site of pJH212, a pPZP212 derivative.

Plant transformation. Gene transfer into plants was performed with Agrobacterium tumefaciens (Strain C58C1, pGV2260, (Deblaere et al., 1985) Solanum tuberosum was transformed according to the method described (Rocha-Sosa et al., 1989) with small modifications. Regenerated plants were screened by PCR for integration of the construct using NPTII and StSUT4 primers (primer sequences: NPTIIa: ACCGGATCTGGATCGTTTCG NPTIIb: TGGTGCCCTATTTGGAACC StSUT4-RNAi: GAGACTCGAGTGCAAGATCTTTGGAACC intron out rev: GATGATTATGTATAACAACG). Plants containing the integrated DNA were amplified in tissue culture and placed in the greenhouse for further analysis. Experiments were carried out with either in vitro propagated clones or from tubers regenerated plants.

Plant growth conditions and tissue culture. Potato plants in sterile culture were grown on 2MS-medium (MS-medium according to Murashige et al., 1962 with 2%
sucrose) in tissue culture chambers at 24°C, at 50% humidity and 1000 μmol photons m\(^{-2}\) sec\(^{-1}\) with a light/dark cycle of 16 h/8 h. Following transformation, leaf discs were put on 2MS with 1 µg/l naphyl acetic acid and 0.1 µg/l benzyl amino purine. For the selection of transformant tissue, 3MS with 2 µg/l zeatin and 35 µg/l kanamycin was used. The root induction of plantlets was performed on 2MS with 2 µg/l indol butyric acid and 50 µg/l kanamycin. After 2 weeks, plantlets were placed on 2MS containing 50 µg/l kanamycin.

**In vitro tuberization assay.** Stem segments including at least one node of 6 weeks old sterile potato plants were prepared under sterile conditions and planted on MS medium containing 10% sucrose. After one week under LD conditions in the growth chamber (16 h light, 8 h darkness, 24°C), the scions were transferred into darkness to induce tubers. *In vitro* tubers were harvested after 20 days.

**Greenhouse.** Transgenic plants were amplified in tissue culture and 60 plants were transferred to soil and grown in a cycle of 16h light (22°C) and 8h darkness (15°C) in 60% humidity. The mean photosynthetic photon flux density (PPFD; 400 – 700 nm) was about 150 μmol photons m\(^{-2}\) sec\(^{-1}\) and additional illumination was provided by high-pressure sodium lamps SON-T Green Power and metal halide lamps MASTER LPI-T Plus (Philips Belgium, Brussels). Emitted light from Philips SON-T Green Power has a red: far-red ratio (660/730 nm) of 2.63 and from Philips HPI-T Plus of 1.25. Both lamps are distributed equally in the green house.

Experiments were repeated independently using either *in vitro* propagated clones of the transformants or potato tubers. Determination of internode elongation was performed as described elsewhere (Martinez-Garcia et al., 2001; Martinez-Garcia et al., 2002). Shading experiments were performed at a plant density of 21 plants * m\(^{-2}\) (shaded plants) and compared to plants grown at a density of 7 plants * m\(^{-2}\) (non-shaded plants). The PPFD’s were determined with a LI-189 (LI-COR, Lincoln, NE, USA) at the level of the investigated leaves and amounted 380 ± 54 (upper leaves of shaded plants), 11 ± 4 (lower leaves of shaded plants), 430 ± 145 (upper leaves of non-shaded plants) and 150 ± 28 μmol photons * m\(^{-2}\) * sec\(^{-1}\) (lower leaves of non-shaded plants). The red:far-red ratio was determined with a Spectroradiometer FieldSpec Pro II FR (with integrated Remote Cosine Receptor) (Analytical Spectral Devices, Inc., Boulder, Colorado, USA). The ratio 660/730 nm was 0.3 ± 0.1 (lower leaves of shaded plants), 1.8 ± 0.1 (upper leaves of shaded plants), 1.5 ± 0.2 (lower leaves of non-shaded plants) and 2.1 ± 0.2 (upper leaves of non-shaded plants).
Thus, far-red light exceeded red light at least threefold during shading experiments. Dark samples were taken under a green light source in the phytochamber. Phytohormone treatment was performed over a 2 weeks period by spraying the plants with 20 µM GA₃ solution supplied with 2 drops of Triton X-100 per liter. Control plants were sprayed with water containing 2 drops of Triton X-100. Ethephone and paclobutrazol was supplied in a concentration of 350 µM, AgNO₃ as a 1 mM solution. All chemicals are purchased from Sigma-Aldrich.

**Artificial shade experiment.** Plants were grown for three weeks in a phytochamber (Heraeus) at 24°C under LD conditions with a white light source (Osram L36W-31) and an additional far red light source (Chopper light type 730 supplied with a Hama 730 nm filter, Chopper Light GmbH, Berlin). Control plants were exposed to white light alone. The PPFD was 290 µmol photons * m⁻² * sec⁻¹ as determined with a LI-189 (LI-COR, Lincoln, NE, USA). The experiment was performed with WT and three different transgenic lines (n=3 per plant line).

**Grafting.** Plants had 4-5 leaves in total when grafted. The experiment was performed as described elsewhere (Martinez-Garcia et al., 2001; Martinez-Garcia et al., 2002).

**Analysis of enzyme activities and determination of soluble sugars.** Soluble sugars and starch were quantified in potato leaf samples extracted with 80% ethanol and 20 mM HEPES-KOH, pH 7.5 as described previously (Hackel et al., 2006)

**RNA Quantification by real-time PCR.** RNA was isolated from different organs of greenhouse grown *S. tuberosum* Désirée and andigena or from leaf discs of potato plants grown in the phytochamber. RNA extraction was performed with Trisure (Bioline, Luckenwalde, Germany) or peqGold Trifast (Peqlab, Erlangen, Germany) according to the manufacturer’s protocol. Reverse transcription was performed with the Qiagen Omniscript RT Kit according to the manual. Optimised conditions included using oligo(dT) primers for the initial reverse transcription reaction on approximately 1 µg of total RNA after digestion with RNase-free DNase (Qiagen, Hilden, Germany). Aliquots of 0.2 µl of the 10 µl RT-reaction were used for the subsequent PCR reaction in the presence of SYBR Green with HotGoldStar DNA Polymerase (Eurogentec, Seraing, Belgium) in a Rotor Gene 3000 Cycler (LTF Labortechnik, Wasserburg, Germany) using the Rotor Gene Software Version 4.6.94. The best products were obtained with the following program: denaturation at 95°C for 30 sec, annealing for 30 seconds at 61°C and elongation for 30 sec at 72°C, in a program of 45 cycles in 20 µl reaction volume. Relative quantification of transcript amounts was
always calculated in relation to the respective ubiquitin transcript level and given in % of ubiquitin. Primers were designed to obtain a 50-150 bp amplicon using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Primer sequences used for real time PCR analysis: Ubiquitin fw: CAC CAA GCC AAA GAA GAT CA, Ubiquitin rev: TCA GCA TTA GGG CAC TCC TT; LC-SUT1 fw: TTC CAT AGC TGC TGG TGT TC; LC-SUT1 rev: TAC CAG AAA TGG GTC GCA AA; StSUT2 fw: GGC ATT CCT CTT GCT GTA ACC; StSUT2 rev: GCG ATA CAA CCA TCT GAG GGT AC; StSUT4 fw: GCT CTT GGG CTT GGA CAA GGC; StSUT4 rev: GGC TGG TGA ATT GCC TCC ACC; PhyB fw: TTT GCC TGA TGC TGG GTA TC; PhyB rev: CTT TGC ACC ACC CCA CTT TA; GA20ox1 fw: CAA GAT TGT GTT GGC GGA CT; GA20ox1 rev: ACT GCT CTG TGC AGG CAA CT; PhyA fw: TGC TCA CTC TCG TGG AGG AT; PhyA rev: TCG TGC AAT GCT AAT TCC AA; StACO3 fw: GTG AGG CCA TCA TTT CTC CA; StACO3 rev: CTT GAA AGC GGA GGT GAC AG. Real time PCR data were corrected by calculation of the PCR efficiency individually using the LinReg PCR software. Statistical analysis was performed with a student’s t-test with 0.05>p>0.01 (+), 0.01>p>0.001 (++) and 0.001>p (+++).

**Western Blot Analysis.** Isolation of the microsomal fraction from plant material as well as two-phase partitioning and western blotting was performed as previously described (Lemoine et al., 1996). The StSUT4-specific peptide antibody is raised against a central loop peptide of SUT4 (NH2-CGSSHTGEEIDESHHGQEEAFLW-CONH2). The specificity of the affinity-purified antibody has been tested previously and the purified antibody was used for immunolocalisation as well as western blot analysis (Weise et al., 2000).

**Acknowledgments**

We gratefully acknowledge Hanjo Hellmann for helpful discussion and Sutton Mooney for english corrections. We thank Yvonne Pörs for setting the light conditions for shading experiments and Dieter Oellerich for setting the artificial shade experiment. We acknowledge Karin Schumacher, Tübingen for providing material. We thank Aleksandra Hackel for excellent technical assistance and Angelika Pötter for the excellent care of green house plants. This work was supported by grants from the DFG (SFB 429) to C.K., P.G. and J.M. and a stipend according to NaFöG to I.C.
References:


Table 1. Tuber yield of potato WT and StSUT4-RNAi plants (*S. tuberosum* Désirée) in g fresh weight after 3 month of growth in the greenhouse under LD conditions. Mean values of four independent experiments are represented (with n= 3 to 11 plants per line and experiment), the standard deviation is given. The tuber yield of StSUT4-RNAi plants is significantly increased (with p<0.05).

<table>
<thead>
<tr>
<th>Plant line no</th>
<th>Tuber yield (g FW)</th>
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<th>Significance</th>
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<td>(+)</td>
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Figure legends

**Fig. 1A.** Expression pattern of *StSUT4* in sink and source organs as determined by real time-PCR. *StSUT4* expression increases during flower development and strongest expression is detected in young developing tubers and in mature flowers. **B.** Western Blot analysis of *StSUT4* in leaves of *Solanum tuberosum*. The microsomal fraction (MF) has been loaded in the first two lanes. Plasma membranes (PM) and endosomal membranes (EF) have been separated by two phase partitioning and loaded on SDS-PAGE. In each lane 15 µg of membrane proteins are loaded. *StSUT4* specific peptide antibodies (Weise et al., 2000) detected the *StSUT4* protein in the correct size of 47 kDa only in the plasma membrane fraction. **C,D,F,G.** Expression of *StSUT4*-GFP fusion expressed under the CaMV 35S promoter in a pCF203 derivative in *Agrobacterium tumefaciens* infiltrated tobacco leaves **E.** The same *StSUT4*-GFP construct expressed in infiltrated potato leaves. **C,F,G.** Single scans **D,E.** Overlay projections of confocal z-stacks. GFP is not only detectable at the plasma membrane, but also in a peri-nuclear ring as shown by propidium iodide staining. **F.** *StSUT4*-GFP fluorescence is detectable at the plasma membrane of tobacco cells as well as in peri-nuclear rings **G.** Same cell shown in F with propidium iodide specific filter settings **H.** Yeast cells expressing a LeSUT4-GFP construct under control of the Adh1-promoter in the yeast expression vector 112A1NE. GFP fluorescence is detected at the plasma membrane and in ER stacks surrounding the nucleus. n= nucleus.

**Fig. 2.** Quantification of sucrose transporter mRNA accumulation by real time-PCR analysis in constant light and *StSUT1* transcript quantification in constant darkness. All three known sucrose transporters from potato are expressed diurnally with distinct maxima. *StSUT2* shows peak levels at the beginning of the light period, whereas *StSUT1* and *StSUT4* show maximal transcript accumulation at the end of the light period. Oscillation of transcript amounts continuous even under 72h of constant light. The amplitude of *StSUT1* oscillation strongly decreases in constant darkness. Relative quantification was performed with ubiquitin as internal standard. The standard deviation is given.

**Fig. 3.** Phenotype of *StSUT4*-RNAi-plants **A.** Plants with reduced *StSUT4* expression show early flowering under LD conditions. Désirée WT and *StSUT4*-RNAi<sub>10</sub> plants after 5 weeks in the green house. **B.** Internode elongation of *S. tuberosum* Désirée WT and *StSUT4*-RNAi plants. Quantification of internode elongation is shown in
Internodes of transformed *S. tuberosum* andigena plants are reduced in length in comparison with andigena WT plants. Andigena plants transformed with a *StSUT4*-RNAi construct showing reduced *StSUT4* transcript levels are able to produce tubers even under LD conditions (D), whereas andigena WT does not (E). *StSUT4*-RNAi2/5 shows early flowering compared to *S. tuberosum* andigena WT plants grown under LD conditions (F).

**Fig. 4 A.** *StSUT4*-RNAi Désirée plants flower on average 6 days earlier than WT plants when grown under LD conditions. Flowering was observed with *StSUT4*-RNAi lines 10 (n=8), 81 (n=9) and 38 (n=9) and *Solanum tuberosum* ssp. tuberosum plants (n=23) grown in the greenhouse under LD conditions. B *StSUT4*-RNAi Désirée plants have significantly less leaves at flowering if grown under LD conditions in the greenhouse (n= 6 for each plant line). C. The tuber yield of *StSUT4*-RNAi potato plants is significantly increased under non-inductive LD. All experiments are reproduced at least three times. One representative example is given (n=5-6 for each line and each time point). The standard deviation is given.

**Fig. 5 A.** Flowering behaviour of grafted *S. tuberosum* WT plants under LD conditions when grafted with *StSUT4*-RNAi plants. Early flowering is observed also in WT plants if grafted with *StSUT4*-RNAi plants depending on the presence of source leaves at the root stock. B. Schematic representation of the graft experiments. Plants were regenerated from tubers and grafted after development of the first six leaves. Graft experiments were repeated twice with *Solanum tuberosum* Désirée with six reciprocal grafts from each transgenic line per experiment. C. Tuber yield of grafted *StSUT4*-RNAi potato plants (ssp. tuberosum) grafted on potato WT plants (ssp. andigena) which do not tuberize under LD conditions. Plants were grafted when they have 4-5 leaves and were kept under LD conditions. As a control, transgenic plants were grafted on their own root stock (right: *StSUT4*-RNAi81 grafted on *StSUT4*-RNAi81 and *StSUT4*-RNAi10 grafted on *StSUT4*-RNAi10) and *Solanum tuberosum* ssp. tuberosum WT was grafted on *S. tuberosum* ssp. andigena, which did not lead to tuber production (left: WT tuberosum grafted on WT andigena). The standard deviation is given. and= andigena, tub= tuberosum.

**Fig. 6 A-C.** Content of soluble sugars in source leaves of *StSUT4*-RNAi plants compared to potato WT plants determined enzymatically. At the end of the light period, the transgenic plants show significantly increased glucose (A), fructose (B) and sucrose (C) content per g fresh weight. D. Efflux of sucrose from leaves of WT...
and StSUT4-RNAi plants was determined by exudation in the presence of EDTA. Sucrose exudation was determined enzymatically in intervals of 3 h during the light period from plants kept under LD conditions in the greenhouse. Sucrose efflux from WT leaves shows maxima at the end of the light period, whereas in StSUT4-RNAi plants sucrose efflux remains high even in darkness. E. Sucrose and starch content of in vitro grown microtubers (n=4 for each plant line). Tubers were harvested 20 days after tuber induction in darkness. The standard deviation is given. F. Sucrose content in the shoot apical meristem of potato WT and StSUT4-RNAi plants. Samples were taken at the end of the light period (9 pm). Fresh weight of samples was between 20 and 60 mg. Error bars indicate the standard deviation. Experiments were performed under LD conditions. Note that floral buds of StSUT4-RNAi plants are first detected when plants had 5-6 mature leaves, whereas WT potato plants started transition from the vegetative to the generative phase when they had >10 leaves.

Fig. 7 A. Transcript levels of StSUT4, the GA biosynthetic enzyme GA20ox1 and the ethylene biosynthetic enzyme StACO3 in StSUT4-RNAi plants as determined by quantitative real time-PCR. A. StSUT4 expression in potato leaves treated with phytohormones and phytohormone inhibitors paclobutrazol (inhibition of GA biosynthesis and silver nitrate (inhibitor of the ethylene receptor). StSUT4 expression is inducible by GA3 at the end of the light period and by ethephon treatment over the whole light period. Potato WT plants were treated with 20 µM GA3, 350 µM paclobutrazol, 350 µM ethephon or 1 mM silver nitrate and StSUT4 mRNA was determined by real time PCR analysis relative to the level of ubiquitin transcripts. B. Quantification of the transcripts of the GA biosynthetic enzyme GA20oxidase1 in WT and StSUT4-RNAi plants showing reduced levels of GA20ox1 in source leaves of StSUT4-RNAi plants at the end of the light period if compared to WT levels. C. Quantification of the transcripts of the ethylene biosynthetic enzyme StACO3 in WT and StSUT4-RNAi plants showing significantly reduced levels of StACO3 mRNA levels in StSUT4-RNAi plants at any time. Ubiquitin transcript levels were used as internal standard for relative quantification in all experiments. Experiments were performed with green house plants grown under LD conditions. The standard deviation is given.

Fig. 8 A. In order to reduce the red: far-red ratio for greenhouse grown plants they were planted at a density of 21 plants * m^-2. Wild type plants show shade avoidance response under canopy shade, showing elongated internodes and hyponastic leaf
movement to capture light under crowded conditions (left side). StSUT4-RNAi plants do not show shade avoidance under canopy shade. Neither internode elongation, nor leaf angle adaptation was observed (right side). B. Internode elongation of WT and StSUT4-RNAi potato plants grown under long day and SD conditions or in high density populations under LD conditions. The experiment was reproduced in the greenhouse and in the growth chamber under LD and SD conditions showing the same results in each case. The length of the five upper internodes was measured as described by (Martinez-Garcia et al., 2002). C. Internode elongation of potato plants grown under canopy shade in the greenhouse. Shaded plants were grown at high plant density (21 plants * m$^{-2}$), whereas control plants were grown at low density (7 plants * m$^{-2}$). D. Internode elongation of potato plants grown under artificial light conditions in the phytochamber. Internode length was measured after three weeks of growth under white light or under white light with additional far red light. The standard deviation is given.

**Fig. 9.** GA$_3$ treatment of potato WT and StSUT4-RNAi plants grown under LD conditions in the greenhouse. A. Source leaves were treated with 20 µM GA$_3$ solution over a period of two weeks every two days. Flowering was analyzed after the indicated period of time. B. Tubers were harvested after two month of growth in the greenhouse. Water treated StSUT4-RNAi plants show higher tuber yield than water treated WT plants due to increased tuber size, whereas GA$_3$ treated StSUT4-RNAi plants show higher tuber number and tuber size than the WT control. C. Internode elongation of potato WT and StSUT4 plants treated with GA$_3$ or paclobutrazol. Error bars indicate the standard deviation.

**Fig. 10.** Hypothetical model of StSUT4-mediated interconnection of the photoreceptor and the GA$_3$ signaling pathway triggering tuberization, flowering and shade avoidance response. The model is partially adapted from Rodriguez-Falcon et al., (2006).
Figure 2 StSUT1

StSUT2

StSUT4

StSUT1

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Figure 3

A

B

Désirée
WT

StSUT4
RNAi
2/5

C

SISUT4
RNAi
2/5

Andigena
WT

F

SISUT4
RNAi
2/5

Andigena
WT

Désirée
WT

StSUT4
RNAi
10

SISUT4
RNAi
25

www.plantphysiol.org
Figure 4A

Day of first flower

(+ +)

(+ +)

(+ +)

WT 10 14 38 63 81 transgenic line no

Day of first flower

WT 10 14 38 63 81 transgenic line no

B

Number of leaves at flowering

(+ +)

(+)

(+ +)

wt StSUT4-RNAi 10 StSUT4-RNAi 38 StSUT4-RNAi 81 plant line no

Number of leaves at flowering

wt StSUT4-RNAi 10 StSUT4-RNAi 38 StSUT4-RNAi 81 plant line no

C

Tuber yield (g FW)

StSUT4-RNAi 10 wt

Tuber yield (g FW)

StSUT4-RNAi 10 wt

Weeks after transfer in the greenhouse

9 11 13 weeks after transfer in the greenhouse

Weeks after transfer in the greenhouse

9 11 13 weeks after transfer in the greenhouse
Figure 5

The bar chart illustrates the day of first flower for different conditions: WT, RNAi, WT+leaves, RNAi-leaves, WT-leaves, and WT+leaves. The error bars indicate the standard deviation.

The other chart shows the tuber yield (g) for various genotypes: WT andig n=5, 81 WT andig n=5, 10 WT andig n=3, WT andig/gs n=4, WT andig/10 n=1, 61/61 n=3, 10/10 n=2. The bars represent the mean tuber yield, with error bars indicating the standard deviation.
A glucose

B fructose

C sucrose

D

E

F

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Figure 9

A

Day of first flower

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B

WT  SUT4-RNAi10  SUT4-RNAi36  SUT4-RNAi63  SUT4-RNAi81

H2O

GA3

C

Length of five upper internodes (cm)

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Figure 10

Day length

CRYs? PHOTs? PHYs

StSUT4 StSUT1

StFT

Ethylene

StCOL3

Sucrose

GA3

Stem elongation

Tuberisation

StFT

Flowering

Ethylene via simulate flow?

LD SD

SD LD

? + +