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Regulation of RhSUC2, a sucrose transporter, is correlated with the light control of bud burst in *Rosa* sp.

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ABSTRACT

In roses, light is a central environmental factor controlling bud break and involves a stimulation of sugar metabolism. Very little is known about the role of sucrose transporters in the bud break process and its regulation by light. In this study, we show that sugar promotes rose bud break and that bud break is accompanied by an import of sucrose. Radio-labelled sucrose accumulation is higher in buds exposed to light than to darkness and involves an active component. Several sucrose transporter (*RhSUC1*, 2, 3 and 4) transcripts are expressed in rose tissues, but *RhSUC2* transcript level is the only one induced in buds exposed to light after removing the apical dominance. *RhSUC2* is preferentially expressed in bursting buds and stems. Functional analyses in baker's yeast demonstrate that *RhSUC2* encodes a sucrose/proton co-transporter with a K_m value of 2.99 mM at pH 4.5 and shows typical features of sucrose symporters. We therefore propose that bud break photocontrol partly depends upon the modulation of sucrose import into buds by RhSUC2.

Key-words: axillary bud; photocontrol; photomorphogenesis; sink strength; sugar loading.

INTRODUCTION

The regulation of sugar transport, allocation and mobilization represents a major event in plant adaptation to environment. In most higher plants, sucrose is the major carbohydrate for long-distance transport through the vascular system (Zimmermann & Ziegler 1975). Sucrose partitioning between its site of synthesis (source tissues), the leaf mesophyll and sink tissues depends on equilibrium between organs. Among them, buds are actively growing heterotrophic organs, whose development depends upon their capacity to import and metabolize sugars (Cottignies 1986; Rinne, Tuominen & Junttila 1994; Maurel *et al.* 2004a,b).

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^{1,2,4}IFR 149 QUASAV – Qualité et Santé des Végétaux.

Previous work in ligneous plants demonstrated that bud burst is accompanied by mobilization of stem carbohydrates: starch in *Quercus robur* (Alaoui-Sossé *et al.* 1994), mainly hexoses in *Prunus persica* (Maurel *et al.* 2004a) and sucrose in *Juglans regia* (Decourteix *et al.* 2008). In *Rosa* sp., bud outgrowth is associated with mobilization of local and distant (surrounding stem portion) sucrose reserves (Girault *et al.* 2010). These findings are in accordance with the stimulation of both sugar (glucose and sucrose) uptake (Marquat *et al.* 1999; Maurel *et al.* 2004a) and the activity of plasma membrane H⁺-ATPase (Aue *et al.* 1999) in bursting buds of *Prunus persica*. Despite all this research, little is known about the involvement or the regulation of soluble sugar transport in bud outgrowth.

Plant architecture is strongly determined by the capacity of buds to grow after release of apical dominance. In several species including *Rosa* sp., bud outgrowth is a complex process involving cell division, new organogenesis and elongation (Zieslin & Halevy 1976; Khayat & Zieslin 1982; Marcelis-van Acker & Scholten 1995; Girault *et al.* 2008), and is controlled by a genetic program and environmental cues. In *Rosa* sp., much evidence showed that light (in quantity and quality) is a powerful factor modulating bud outgrowth (Healy & Wilkins 1980; Mor, Halevy & Porath 1980; Khayat & Zieslin 1982; Girault *et al.* 2008). Light triggers a high bud sink strength through sugar accumulation (sucrose and glucose), concomitantly with an increase in both transcript levels and enzymatic activity of acid vacuolar invertase in buds (Girault *et al.* 2010). Neither sucrose accumulation nor sugar metabolism stimulation was found to occur in the dark, where bud burst was totally prevented (Girault *et al.* 2010). Thus, light induction of bud burst is likely to require higher sucrose import, through an up-regulation of sugar transporters.

Sugar transport at the cellular level follows either the symplastic pathway (through the plasmodesmata) or the apoplastic pathway, which is mediated by plasma membrane-localized sugar transporters. These proteins, known as sugar transporters or sugar carriers, mainly catalyse active energy-dependent transport (Delrot *et al.* 2001; Kühn *et al.* 2003). They are trans-membrane proteins, with 12 characteristic loops of the major facilitator superfamily

(MFS) (Lemoine 2000). Many sucrose transporter (named SUC or SUT) genes have been so far isolated from herbaceous and woody plants such as *Betula pendula* (Wright *et al.* 2000), *Citrus* sp. (Li *et al.* 2003), *Hevea brasiliensis* (Dusotoit-Coucaud *et al.* 2009; Tang *et al.* 2010), *Juglans regia* (Decourteix *et al.* 2006), *Malus domestica* (Fan *et al.* 2009) and *Vitis vinifera* (Davies, Wolf & Robinson 1999; Ageorges *et al.* 2000).

Genome sequencing demonstrated that sucrose transporters are encoded by a small multigenic family: five genes in *Oryza sativa* (Braun & Slewinski 2009) or nine genes in *Arabidopsis thaliana* (Baud *et al.* 2005). In this latter, seven genes encode functional sucrose transporters, whereas two genes (*AtSUC6* and *AtSUC7*) code aberrant and non-functional proteins (Sauer *et al.* 2004). Based on their sequence homology and biochemical activity, SUTs were assigned into either three (Aoki *et al.* 2003), four (Sauer 2007) or five groups (Braun & Slewinski 2009; Kühn & Grof 2010). Most of these sucrose transporters are localized in the plasma membrane, while some of them are found in the tonoplast (Sauer 2007). All plasma membrane sucrose transporters have been identified as energy-dependent proton sucrose/H⁺ transporters, except the sucrose facilitators (SUFs) isolated from *Pisum sativum* and *Phaseolus vulgaris*. SUFs catalyse bidirectional sucrose transport in a pH- and energy-independent manner (Zhou *et al.* 2007).

SUTs are involved in: (1) loading sucrose into the phloem from the apoplast of the source cell; (2) maintaining a high sucrose concentration in the phloem; and (3) unloading to sink cells (Williams, Lemoine & Sauer 2000 for review). Their role in diverse sink tissues has been well documented in the literature. For example, in potato tuber, specific antisense repression of *SUT1* was accompanied by a reduction of phloem unloading (Kühn *et al.* 2003); fertility of tomato fruits was highly reduced in *LeSUT2* antisense plants (Hackel *et al.* 2006); in grape, *VvSUC27* is highly expressed after flowering (Davies *et al.* 1999; Zhang *et al.* 2008). Although the bursting bud is considered an active sink organ and requires a high sugar import to satisfy its carbon demand, very little is known on the involvement of this kind of plasma membrane transporter in this morphogenetic process.

Sucrose transporter expression is highly regulated at both transcriptional and post-transcriptional level (Williams *et al.* 2000; Kühn & Grof 2010 for review). Wounding (Meyer *et al.* 2004), salt stress (Noiraud, Delrot & Lemoine 2000), temperature (Decourteix *et al.* 2006) and light (Shakya & Sturm 1998; Matsukura *et al.* 2000; Chincinska *et al.* 2008; Kühn & Grof 2010) evoked mRNA accumulation. Furthermore, these genes were also regulated by endogenous factors such as sugar concentration (Weber *et al.* 1997; Chiou & Bush 1998; Li *et al.* 2003; Tang *et al.* 2010) or the phytohormones, gibberellins (Chincinska *et al.* 2008; Chen *et al.* 2010), ethylene (Dusotoit-Coucaud *et al.* 2009) and cytokinins (Ehness & Roitsch 1997). These changes may result from direct action of physical or biochemical signals, or indirectly through modulation of sugar concentrations in cells.

In *Rosa* sp., previous data from our laboratory involved light as an essential environmental factor targeting the bud itself to trigger its development in a beheaded plant system (Girault *et al.* 2008). This light-mediated bud burst is concomitant with the stimulation of sugar metabolism through an up-regulation of a vacuolar acid invertase (Girault *et al.* 2010) and local accumulation of sugars (sucrose and glucose). In addition, the essential need of light for bud burst is specific to *Rosa* sp., and was not found in any tested model plants such as *Arabidopsis*, poplar or tomato (Girault *et al.* 2008). Within this context, the determination of the mechanism of sucrose unloading in rose buds is a first step towards the understanding of the mechanism by which light modulates bud break and ultimately plant architecture.

This work aimed to provide insight into the role of sucrose transporters in the photocontrol of bud burst. Here, we present sucrose transporter isolation, their expression in buds exposed to light/dark conditions and their tissue-specific expression pattern. We determined the sucrose uptake kinetics of one of them, RhSUC2, using a baker's yeast system. Its active role in bud sucrose import to promote bud burst under light in *Rosa* is discussed.

MATERIALS AND METHODS

Plant material and growth conditions

Plants (*Rosa hybrida* 'Radrazz') were propagated by cuttings from a single plant genotype as previously described (Girault *et al.* 2008). Experiments were performed on plants displaying an emerging flower bud, hereafter referred to as a 'visible flower bud' (VFB). At this stage, the plant bore at least seven leaflet leaves, and all axillary buds remained dormant because of the apical dominance exerted by the shoot apical meristem. In all experiments, we studied axillary bud of the third proximal leaflet leaves (named 'bud of interest'). For this study, two experimental models were used:

- 1 Beheaded plants (plants with apical part removed; same plant model as Girault *et al.* 2010): Plants were grown at 25 ± 3 °C under white light (100 ± 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$, L : D 16:8 photoperiod, fluorescent tubes Osram L36W/77; Fluora, München, Germany) or under total darkness. The dark condition was generated by wrapping the plant with light-proof plastic foil, as described by Girault *et al.* (2008). Plants were placed under these growth conditions for 6 d (for morphological analyses) or for 1–4 d (for gene expression). Non-beheaded (intact) plants were used as control.
- 2 *In vitro* single node cuts: The stem portion bearing the bud of interest and the neighbouring region (1 cm both side) was cut, and the leaves and thorns were gently removed. Stem portions were soaked in water to avoid vessel cavitation, and subsequently sterilized with 70% ethanol. The stem segments were placed on the basic culture media (Murashige & Skoog 1962), supplemented with 8 g L⁻¹ Aubygel G3 agar and 0.2% Plant

Preservative Mixture (Kalys, Bernin, France). Various amounts of sugars (mannitol, sucrose or sorbitol) were added to a final concentration of 0, 100, 250 or 500 mM. Stem segments were placed in a culture chamber (KBW720; Binder, Tuttlingen, Germany), under controlled conditions: constant temperature (23 °C), total darkness or $125 \pm 25 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light (L : D 16:8 photoperiod) during 6–12 d (morphological analyses) or during 72 h (sugar content analyses). To test the effect of auxin on sucrose transporter expression, stem portions were grown on Murashige and Skoog (MS) media, containing 250 mM glucose with 0, 0.1, 1 or 10 μM of *N*-(1-naphthyl) acetic acid (NAA). Gene expression analyses were performed after 72 h of auxin treatment under $125 \pm 25 \mu\text{mol m}^{-2} \text{s}^{-1}$ white light (L : D 16:8 photoperiod) at 23 °C.

Morphological analyses

After 6 d of light/dark treatment, the length of the distal bud was measured using a digital caliper (0–150 mm) and the total number of leaf-like organs (including scales, preformed leaves at different development stages and leaf primordia) was determined after manual dissection of the bud, under a stereo-dissecting microscope.

Sugar content analyses

Sugar content analyses were performed using 40 mg of frozen tissues. Tissues were ground to a fine powder and homogenized with 400 μL of extraction buffer [45 mM HEPES (pH 7.0), 9 mM MgCl_2 , 0.9 mM ethylenediaminetetraacetic acid (EDTA), 2.6 mM dithiothreitol (DTT), 10% ethylene glycol, 0.02% Triton X-100] and centrifuged 3 min at 12 000 *g* (4 °C). The supernatant was collected and used for sugar (starch, sucrose, D-glucose and D-fructose) content determination using a Konelab 20i sequential automat (Thermo Electron, Vantaa, Finland) and the ENZYTEC system (SCIL Diagnostics, Martinsried, Germany). Analyses were performed on three independent biological samples.

¹⁴C sucrose accumulation analyses

Bud sugar accumulation analyses were carried out on buds harvested from beheaded plants grown as previously described. After harvesting, buds were washed for 30 min in 300 μL of control solution (100 mM mannitol, 20 mM MES, 0.25 mM CaCl_2 , 0.25 mM MgCl_2 , pH 5.5) with or without 0.5 μM CCCP (Sigma, Évry, France). Buds were then soaked and transferred to solid control media supplemented with 1 mM [¹⁴C] sucrose (0.5 $\mu\text{Ci mL}^{-1}$, 21.8 kBq mmol^{-1} , Perkin Elmer, Les Ulis, France) and 0 or 0.5 μM CCCP. The sectioned part of bud was the only region directly in contact with the labelled media. The incubation was performed for 2 h, then buds were washed three times for 5 min with 500 μL of control solution. Tissues were treated with 300 μL of digesting solution (Triton 0.1X, 13% HClO_4 , 12% H_2O_2) for 12 h at 55 °C. Four millilitres of liquid scintillation

cocktail (Ecolite(+)TM liquid scintillation fluid; MP Bio-medicals, Paris, France) was added and the radioactivity measured in a liquid scintillation counter (Packard, Tricarb 1900TR, Les Ulis, France). Sugar uptake was calculated from the radioactivity measurements. Each experiment was done three times with three to five pooled buds. Buds grown under darkness were kept under darkness during sugar uptake assays.

RNA extraction and cDNA synthesis

Buds were collected from the two experimental models (beheaded plant and *in vitro* single node explant), frozen in liquid nitrogen and stored at –80 °C until use. For plants grown in darkness, tissue collection was performed under green light. Frozen tissues (60–100 mg) were ground using mortar, pestle and liquid nitrogen in the presence of 10% PVP40 (w/w). Total RNA was then isolated using NucleoSpin RNA Plant Kit (Macherey-Nagel, Paris, France), its quantity evaluated with a NanoDrop spectrophotometer (Fisher Scientific, Illkirch, France) and its quality checked by gel electrophoresis.

RNA (1 μg) was reverse transcribed with oligo(dT)₂₀ primer using SuperScript III Reverse transcriptase kit (Invitrogen, Paris, France). Genomic DNA contamination was verified by PCR using specific primer designed against an intronic region of *RhGAPDH* gene. cDNA was purified with QIAquick PCR Purification Kit (QIAGEN, Les Ulis, France) and diluted 400 times with water and stored at –20 °C until needed.

Sequence identification and cloning

A screen of rose genomic resources (URGI, <http://urgi.versailles.inra.fr/> and Genome Database for *Rosaceae* (GDR), <http://www.rosaceae.org>) allowed us to identify a single Expressed Sequenced Tag (*RhSUC3*, CL_RO_3183.1–19612540) that potentially encodes a sucrose transporter. Other putative sucrose transporters were PCR amplified from different tissues using the degenerate primers *RhSUCa* [forward-C(AGCT)TT(CT)TT(CT)A TGGC(GC)GT(GCT)GG/reverse – TC(AT)(CT)(GT)(AGC)CCCATCCA(AG)TC(AGT)GT] and *RhSUCb* [forward – CT(AG)AC(CT)CC(AGCT)TA(CT)GT(ACT)CA(AG)CT/reverse – GG(AG)AACCA(AGCT)GC(AGT)ATCCA(AG)T]. The PCR products were subsequently cloned into pGEM-T-Easy vector and sequenced (Genome Express, Grenoble, France).

The *RhSUC2* full-length cDNA sequence was isolated using RACE-PCR (Marathon cDNA Amplification Kit; Clontech, Paris, France) with cDNA made from buds harvested from beheaded light-grown plants. The *RhSUC2* 3' and 5'-end amplifications were performed with BD Advantage 2 PCR Enzyme System (BD Biosciences, Paris, France) with AP1 and *RhSUC2*-F1 (CCATCTTCGTGGTCTGGG TTCTGGAT) or *RhSUC2*-R1 (GGATACGAGAGCGA GAATGGTGAGGGC) primers. PCR products were then cloned into the pGEM-T-Easy vector and sequenced.

Gene expression analyses

Organ-specific gene expression was assessed on purified cDNA produced from dormant and bursting axillary buds, flower buds, young and mature leaves, stems and roots. Amplifications were performed in standard conditions using 0.5 U of GoTaq Flexi DNA polymerase (Promega, Charbonnières les bains, France) in a final volume of 25 μ L. Reactions were set up with following primer pairs: SUC1 (forward-CGGCTGGATCGCTAAACGTT/reverse-TGAGGTTGGCGCAGTAAATGTC), SUC2 (forward-CTACACCCACCTCCACAAGATG/reverse-ACCAAGGATCTCGCTGAAGA), SUC3 (forward-AGACCGACTCGGTGTCGATTTC/reverse-GCTCATCATCCATCCCATCA) and SUC4 (forward-TTGGCAATATTCTTGGCTATG-CAA/reverse-CGCACGCGGAGGTAA GTGTA), and for calibration SAND1 (forward-TCTCCTCATTTGAACCAACACACA/reverse-AGGGCCACC TACGCCATCAT). PCR products were separated on agarose gel. Experiments were repeated at least three times.

Real-time PCR reactions were performed using the iQ SYBR Green Supermix (Bio-Rad, Marnes-la-Coquette, France) and fluorescence was detected using Chromo4 Real-time PCR Detector (Bio-Rad). Each RT-PCR reaction was done in triplicate on three independent biological samples. Primer efficiency was determined as described by Rasmussen (2001), and the abundance of targeted transcript was normalized with the SAND1 transcript level and expressed relative to the control condition (before beheading) using the following formula: $R = [(E_{\text{target}})^{\Delta\text{CT}_{\text{target}}(\text{control treated})}] / [(E_{\text{reference}})^{\Delta\text{CT}_{\text{reference}}(\text{control treated})}]$ (Pfaffl 2001).

RhSUC2 functional validation

The functionality of RhSUC2 was carried out in baker's yeast (*Saccharomyces cerevisiae*). We used the SUSY7 strain (*suc2::URA3 malO leu2 trp1/YIP128A2-SUSY*) for transformation (Riesmeier, Willmitzer & Frommer 1992). Full-length CDS of *RhSUC2* was amplified from the cDNA library using the following primers: forward (GAGAGAA TTCCAGCAATGGAAGTCGAAACCACCCA) and reverse (GAGACTCGAGCGATCGAAATCAGTGGA ATGCAGCAGCT). PCR product was purified with QIAquick PCR Purification Kit (QIAGEN), digested with EcoRI and XhoI (Promega, Yverres, France) and cloned into digested pDR296 expression vector. This plasmid is derived from pDR195, using TRP1 instead of URA3 as the selection gene (Rentsch *et al.* 1995).

Sucrose uptake assays were performed on transformed yeast (with the empty pDR296 or the *RhSUC2*-pDR296) grown to early logarithmic phase in YNB medium supplemented with 2% glucose. Cells were washed and resuspended with 50 mM MES buffer (pH 4.5) to reach a final OD_{600nm} value of 0.3. The pH dependency study was performed with cells resuspended in distilled water. Aliquots (100 μ L) of cell suspension were incubated in 100 μ L of a solution containing 50 mM MES (pH 4.5), 1 mM [U-14C]

sucrose (0.5 μ Ci mL⁻¹, 18.43 kBq mmol⁻¹, Perkin Elmer) at 28 °C for 1, 2, 5 and 10 min for time course assays. Concentration dependence assays were performed by using the same solution containing 0.2–10 mM [U-14C] sucrose (0.5 μ Ci mL⁻¹) at 28 °C for 2 min. For pH dependency study, cells were incubated with 100 μ L of a solution containing 100 mM MES (pH 4.0–7.0) and 1 mM [U-14C] sucrose (0.5 μ Ci mL⁻¹) at 28 °C for 2 min. Sugar specificity and inhibitor studies were monitored with 100 μ L of a solution containing 50 mM MES (pH 4.5) and 5 mM [U-14C] sucrose (0.5 μ Ci mL⁻¹) added with 50 mM of competing sugars or inhibitors. Cells were incubated for 2 min at 28 °C. The reactions were stopped by adding 8 mL of cold water and immediate filtration on glass microfibre filters (25 mm, Fisher Bioblock, Illkirch, France). This step was repeated once. Radioactivity incorporated into cells collected on filters was evaluated using a liquid scintillation counter.

Statistical analyses

The Student or Mann–Whitney tests were used to compare two conditions. To compare more than two conditions, the Kruskal–Wallis test possibly followed by pairwise comparisons with the Mann–Whitney tests and Bonferroni correction were carried out. The statistical computations were performed using the PAST software (<http://folk.uio.no/ohammer/past/>).

RESULTS

Effect of beheading and sugar supply on rose bud break, elongation and organogenesis under light

Control buds (before treatments) are fully dormant with adherent scales (Fig. 1a). After apex removal, a 6 d light exposure was sufficient to induce the outgrowth of 90% of rose buds (Table 1). At this stage, developed leaves (harbouring leaflets) had just emerged from the bud (Fig. 1b). In contrast, when grown 6 d *in vitro* in the absence of sugars, the majority of buds (81%) were merely swollen and only 19% outgrowth (Fig. 1c). Supplying buds with the non-metabolizable sugar (mannitol) also caused the buds to enlarge (Fig. 1d), but did not induce any outgrowth (0% at 100 and 250 mM, and 6% at 500 mM). In contrast to mannitol, 100 mM sorbitol did induce bud burst (81%), while higher amounts (250 and 500 mM) did not (Fig. 1e). Interestingly, sucrose gave rise to a massive bud burst, with a maximum of 100% at 250 mM, where buds exhibited a similar morphology to those on beheaded plants (Fig. 1f).

Bud elongation and organogenesis were also analysed (Table 1). Buds grown for 6 d in the absence of sugars showed very limited growth compared to plants before treatment and beheaded plants, while the mannitol-treated ones were nearly indistinguishable from plants before treatment. However, a stimulation of organogenesis was found in buds incubated with a high concentration (250 mM) of mannitol. Buds supplied with 100 mM sorbitol showed a partial

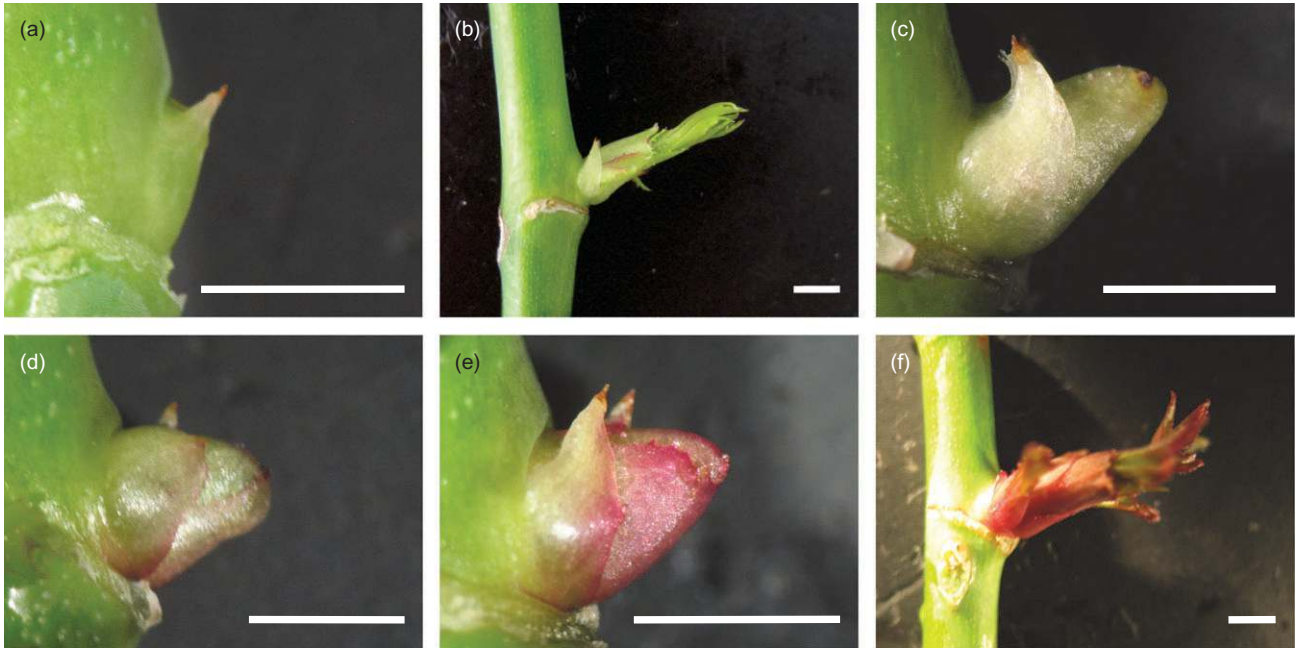


Figure 1. Effect of *in vitro* sugar feeding and plant beheading on bud burst after 6 d under light. Dormant buds before treatment served as control (a). *In planta* buds after beheading (b). Buds grown *in vitro* without sugar (c), with 250 mM mannitol (d), sorbitol (e) or sucrose (f). The horizontal white bars = 2 mm.

stimulation of elongation (2.3-fold when compared to buds grown without sugar), while higher concentrations (250 mM) caused a response similar to that induced by mannitol. Sucrose showed varying responses: 100 and 250 mM stimulated both elongation (5.5- and 5.1-fold), and organogenesis

(one and three additional primordia) when compared to buds grown without sugar, respectively. In addition, 500 mM sucrose caused a weaker stimulation of these two processes (2.2-fold for elongation and no additional primordia was synthesized, when compared to buds grown without sugar).

Table 1. Bud burst, length and leaf-like organ number before treatment, after 6 d of *in vitro* sugar feeding or plant beheading, under light

Treatment	Light			
	Bud burst percentage	Bud length (mm)	Number of leaf-like organ	Number of plants
Before treatment	0	1.8 ± 0.3 (a)	9.3 ± 0.7 (a)	18
Stem portions grown <i>in vitro</i> with:				
0 mM sugar	19	3.3 ± 1.4	12 ± 0.6	16
100 mM				
Mannitol	0	1.9 ± 0.7 (a)	10 ± 0.9 (a)	16
Sorbitol	81	5.3 ± 1.6 (b)	12 ± 1.0	16
Sucrose	89	10 ± 3.4 (b)	13 ± 0.9 (b)	18
250 mM				
Mannitol	0	2.1 ± 0.4 (a)	11 ± 0.8	16
Sorbitol	0	2.1 ± 0.6 (a)	11 ± 0.8	14
Sucrose	100	9.5 ± 1.4 (b)	15 ± 0.7 (b)	16
500 mM				
Mannitol	6	1.6 ± 0.3 (a)	9.4 ± 1.7 (a)	16
Sorbitol	0	1.3 ± 0.2 (a)	9.9 ± 0.8 (a)	16
Sucrose	75	5.1 ± 2.7	13 ± 2.0	16
Beheaded plants	90	7.5 ± 2.8 (b)	12 ± 0.8	30

Plants before treatment are used as controls. Bud length and leaf-like organ values are means ± standard errors of all measurements done for each condition. Letters indicate a significant difference lower (a) or higher (b) compared to buds of stem portions grown without sugars ($P < 0.01$, Student's *t*-test).

Effect of sugar supply on rose bud sugar content under light

Glucose, fructose, sucrose and starch contents were analysed after 72 h (3 d) in buds supplied *in vitro* with 250 mM sugars (Fig. 2). Dormant buds (control) contained high quantities of sucrose ($7.36 \pm 2.46 \text{ mg g}^{-1} \text{ FW}$) and low quantities of glucose ($1.15 \pm 0.23 \text{ mg g}^{-1} \text{ FW}$), fructose ($0.78 \pm 0.11 \text{ mg g}^{-1} \text{ FW}$) and starch ($0.45 \pm 0.21 \text{ mg g}^{-1} \text{ FW}$). Buds grown without sugars exhibited a reduction of all soluble sugar contents (2.2- to 2.3-fold relative to dormant buds), whereas starch content was not significantly reduced. Supplying buds with 250 mM mannitol induced a reduction of their sucrose content (3.4-fold relative to dormant buds), whereas hexose and starch contents were not affected by the treatment. Bud sugar contents remained unchanged relative to control only when buds were supplied with 250 mM sucrose.

Sucrose accumulation into buds of beheaded plants under light and dark conditions

Total radio-labelled sucrose accumulation into buds 96 h after beheading and exposure to light or dark conditions was analysed (Table 2). Dormant buds (control) were able to take up sucrose ($4.62 \pm 0.76 \mu\text{Bq}$ per bud). Sucrose absorption by buds was significantly induced by light ($11.93 \pm 0.98 \mu\text{Bq}$ per bud), but only slightly by dark ($6.80 \pm 2.31 \mu\text{Bq}$ per bud). To check whether sucrose uptake was mediated by an active process, radio-labelled sucrose accumulation into buds was performed in the absence or presence of $0.5 \mu\text{M}$ of CCCP (a protonophore) under light and dark condition. CCCP highly reduced sucrose accumulation into buds exposed to light (56%) and dark (44%) (data not shown). This result indicates that an electrochemical gradient across the plasma membrane is essential for sucrose accumulation into buds.

Isolation of four putative sucrose transporters (*RhSUC1*, 2, 3 and 4)

Data from Unité de Recherche en Génomique et Bio-Informatique (URGI) and GDR databases allowed us to identify a partial cDNA sequence (637 bp), corresponding to the 5'-end of a putative sucrose transporter gene from *R. hybrida*: *RhSUC3* (CF349302) (Guterman *et al.* 2002).

We designed degenerated primers directed against conserved domains of the previously isolated sucrose transporters from several species to identify additional putative sucrose transporters in *R. hybrida*. Three partial cDNA sequences were obtained and referred as to *RhSUC1* (HO762757, 367 bp), *RhSUC2* (755 bp) and *RhSUC4* (HO762758, 397 bp), based on their orthologs in *Arabidopsis thaliana*. Predicted protein sequences of partial and complete isolated *R. hybrida* sucrose transporters were compared with other protein sequences (NCBI/Protein) using ClustalW, and the resulting phylogenetic tree was visualized with Treeview (Fig. 3). *RhSUC1* partial putative

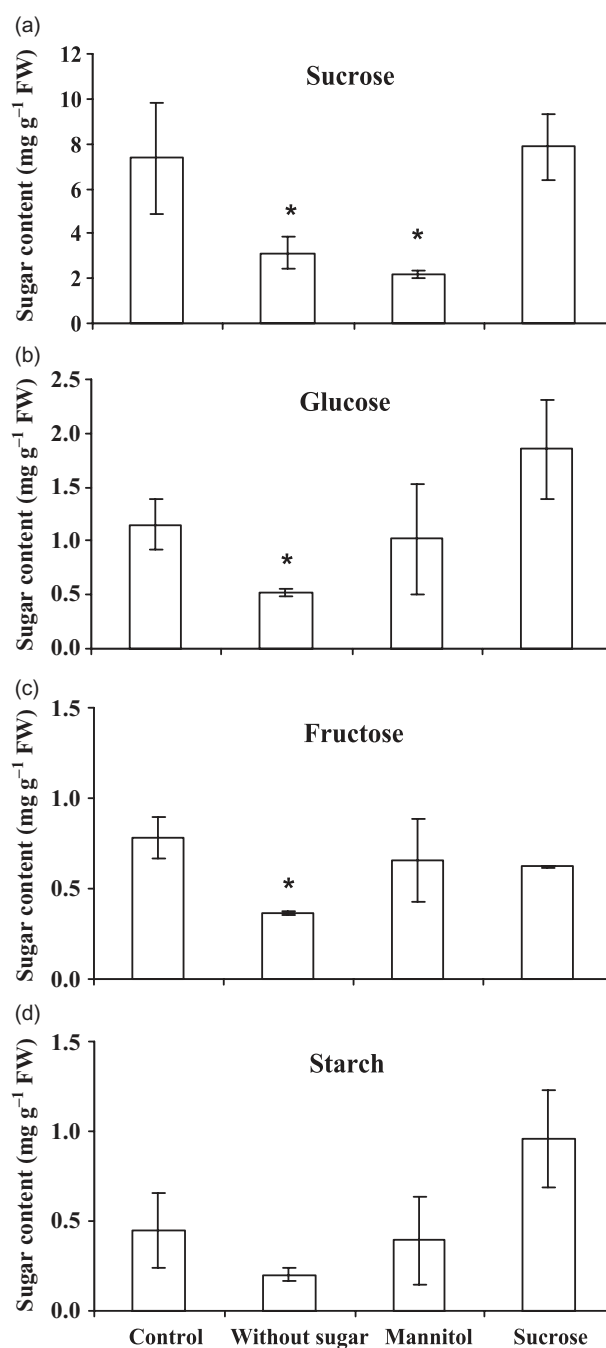


Figure 2. Effect of *in vitro* sugar feeding on bud sugar content, before treatment and after 72 h under light. Buds were grown *in vitro* without sugar, with 250 mM mannitol or sucrose. Dormant buds before treatment served as control. Sucrose (a), glucose (b), fructose (c) and starch (d) contents were determined. Each value is a mean of three independent biological replicates. Error bars represent standard errors. Asterisks indicate significant differences with the control (Student's *t*-test, $P < 0.05$).

protein shares the highest sequence similarity with HbSUT1A (68%) (Dusotoit-Coucaud *et al.* 2009) and RcSCR1 (67%) (Eisenbarth & Weig 2005), and 66% identity with AtSUC1 being its closest sequence in *Arabidopsis*. *RhSUC2* putative protein shares 73, 72, 70 and 66% identity

Table 2. Bud sucrose accumulation after plant beheading and growth under light or darkness

Treatment	Total sucrose accumulation (μBq per bud)
Before beheading (control)	4.62 ± 0.76 (a)
Beheaded plants exposed	
96 h to :	
Light	11.93 ± 0.98 (b)
Darkness	6.80 ± 2.31 (a)

Plants at visible flower bud (VFB) stage were beheaded, their leaves were removed and plants were treated under light or darkness for 96 h. Untreated plants were used as control before treatment. Buds were incubated 2 h in the presence of 1 mM [U- ^{14}C] sucrose (18.5 kBq mL $^{-1}$). Letters indicate statistical groups (Student's *t*-test, $P < 0.05$). Each value is a mean of three independent biological replicates \pm SE.

with the HbSUT1 (A and B) (Dusotoit-Coucaud *et al.* 2009), RcSCR1, JrSUT1 (Decourteix *et al.* 2006) and AtSUC2 (Sauer & Stolz 1994) sucrose transporters, respectively. The full-length *RhSUC2* cDNA (HQ403679, 1982 bp) encodes a putative protein of 513 amino acids with a calculated molecular mass of 54.6 kDa. It presents several characteristics common to the previously characterized plant sucrose transporters belonging to the MFS (Lemoine 2000; Saier 2000). It harbours cytoplasmic N- and C-terminal extremities, and a central cytoplasmic loop between transmembrane segments VI and VII (Sauer 2007). RhSUC1 and RhSUC2 belong to the group 2 (Sauer 2007), which is localized in the plasma membrane of dicotyledons. RhSUC3 partial putative protein shows highest similarity with HbSUT2 (B and C) (66%) (Dusotoit-Coucaud *et al.* 2009). Its closest sequence in *Arabidopsis* (*AtSUC3*) belongs to the third group of plant sucrose transporters that are easily differentiated from others by an extension of their N-terminal extremity and their central cytoplasmic loop. The closest relatives of RhSUC4 partial putative protein are HbSUT4 (Dusotoit-Coucaud *et al.* 2009), MdSUT1 (Fan *et al.* 2009) (77%) and AtSUC4 (Weise *et al.* 2000) (64%). It belongs to the group 4 of plant sucrose transporters that are mainly expressed in sink tissues and at least some of them are proposed to be localized in the tonoplast.

***RhSUC1, 2, 3 and 4* expression in *R. hybrida* organs**

The putative sucrose transporters differ greatly in their expression pattern in the tested organs (Fig. 4). *RhSUC1* transcript is mainly accumulated in stems, and to a much lower extent in mature leaves and flower buds. *RhSUC2* strongly accumulated in bursting buds and in stems. This transcript also showed a slight accumulation in young leaves. No accumulation of *RhSUC2* was found in mature leaves, suggesting its expression is specific to sink tissues. *RhSUC3* and *RhSUC4* showed a low, but constitutive transcript accumulation in all tested organs.

Transcript pattern of *RhSUC2, 3 and 4* into buds of beheaded plants under light and dark conditions

The relative gene expression of *R. hybrida* sucrose transporters was assessed on beheaded plants grown under light or total darkness from 24 to 96 h (Fig. 5). Untreated plants were used as control. No expression of *RhSUC1* was detected in buds under light or dark conditions. *RhSUC2* was the only transporter displaying significant difference in transcript accumulation in buds between light and dark conditions from 48 to 96 h after beheading. Its transcript level was induced (3.5- to 4-fold) within 24 h after beheading, under light and darkness. This induction was stable in light over the time course (until 96 h), but not in darkness where *RhSUC2* transcript level dropped and returned to its basal level 48 h after beheading.

RhSUC3 and *RhSUC4* transcript levels were unaffected by beheading, or by the presence or absence of light over the time course frame (until 96 h).

Functional characterization of *RhSUC2* in baker's yeast

As *RhSUC2* expression is correlated to the light-induced bud break, its transport activity was tested in a heterologous system, baker's yeast. We expressed *RhSUC2* cDNA in the SUSY7 yeast strain, which is deficient in both cell wall invertase and maltose utilization, and expresses a potato sucrose synthase (Riesmeier *et al.* 1992). Our data (Fig. 6; Table 3) demonstrate that *RhSUC2* encodes a functional sucrose/H $^{+}$ co-transporter.

Sucrose uptake of *RhSUC2* cDNA complemented cells was clearly higher (31.5-fold after 10 min) than those complemented with the empty pDR296 vector. RhSUC2 sucrose uptake was constant and did not level off during the 10 min time course. It was pH dependant with an optimum at pH 4.5 (Fig. 6b). The protonophore CCCP and the -SH group blocker PCMBs that are well characterized as sucrose/H $^{+}$ transporter inhibitors caused a massive drop in sucrose uptake (75 and 91%, respectively relative to the control Table 3). The affinity of RhSUC2 for sucrose was also investigated at pH 4.5 for sucrose concentrations ranging from 0.1 to 5 mM (Fig. 6c). A Lineweaver-Burk transformation of data revealed, at this pH, that RhSUC2 displayed a K_m value of 2.99 mM and a V_{max} of 21.4 nmol sucrose mg $^{-1}$ protein min $^{-1}$ (Fig. 6d). Interestingly, RhSUC2 sucrose transport activity was repressed (63%) in the presence of 5 mM sucrose, a concentration close to the K_m value.

The substrate specificity of RhSUC2 was assessed in the presence of an excess of various unlabelled sugars (25 mM) (Table 3). Among all tested sugars, sucrose and maltose were the only ones to compete with ^{14}C -sucrose uptake (reduction of 79 and 57% of sucrose uptake activity when compared to control, respectively). In contrast, glucose and fructose stimulated sucrose uptake.

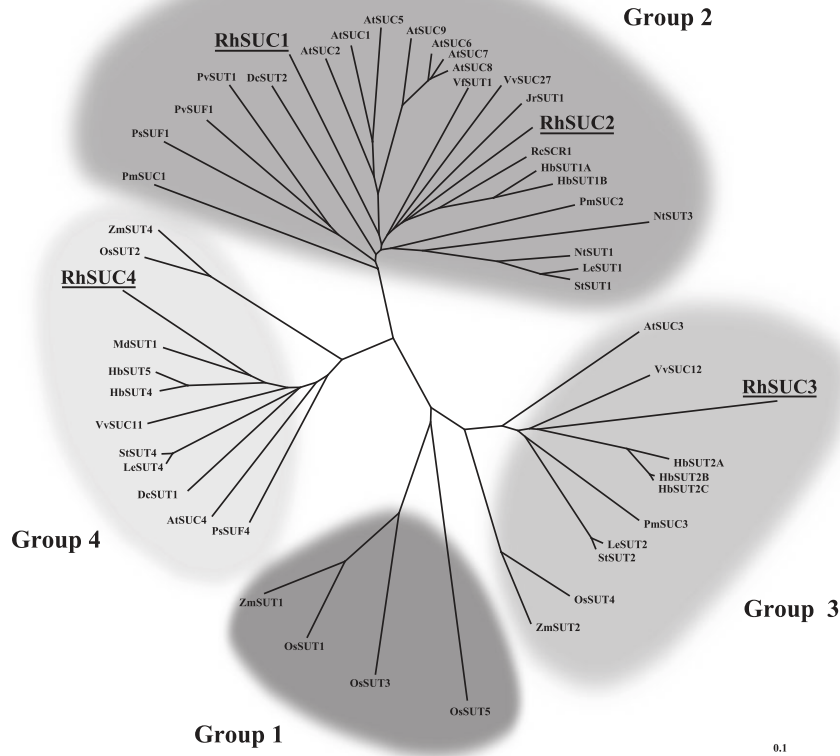


Figure 3. Phylogenetic tree of plant sucrose transporters. Confirmed or predicted plant sucrose transporter complete protein sequences (NCBI/Protein) were used to construct a phylogenetic tree. Partial or complete *Rosa hybrida* sucrose transporter predicted protein sequences were compared to sucrose transporters from other plant species using ClustalW. Phylogenetic tree was visualized with Treeview. Phylogenetic groups defined by Sauer (2007) are indicated. Accession numbers of presented sucrose transporter sequences are: AtSUC1 (*Arabidopsis thaliana*; NP_177333), AtSUC2 (NP_173685), AtSUC3 (NP_178389), AtSUC4 (NP_172467), AtSUC5 (NP_177334), AtSUC6 (NP_199174), AtSUC7 (NP_176830), AtSUC8 (NP_179074), AtSUC9 (NP_196235), DcSUT1 (*Daucus carota*, Y16766), DcSUT2 (Y16768), HbSUT1A (*Hevea brasiliensis*, DQ985466), HbSUT1B (AM492537), HbSUT2A (DQ985467), HbSUT2B (DQ985465), HbSUT2C (AM491808), HbSUT4 (EF067335), HbSUT5 (EF067333), JrSUT1 (*Juglans regia*, AAU11810), LeSUT1 (*Lycopersicon esculentum*, CAA57726), LeSUT2 (AAG12987), LeSUT4 (AAG09270), MdSUT1 (*Malus × domestica*, AAR17700), NtSUT1 (*Nicotiana tabacum*, X82276), NtSUT3 (AAD34610), OsSUT1 (*Oryza sativa*, AAF90181), OsSUT2 (Q0ILJ3), OsSUT3 (BAB68368), OsSUT4 (BAC67164), OsSUT5 (BAC67165), PmSUC1 (*Plantago major*, CAI59556), PmSUC2 (X75764), PmSUC3 (CAD58887), PsSUF1 (*Pisum sativum*, ABB30163), PsSUF4 (ABB30162), PvSUF1 (*Phaseolus vulgaris*, ABB30165), PvSUT1 (ABB30164), RcSCR1 (*Ricinus communis*, CAA83436), RhSUC1 partial (*R. hybrida*, HO762757), RhSUC2 (HQ403679), RhSUC3 partial (CF349302), RhSUC4 partial (HO762758), StSUT1 (*Solanum tuberosum*, CAA48915), StSUT2 (AAP43631), StSUT4 (AAG25923), VfSUT1 (*Vicia fava*, CAB07811), VvSUC11 (*Vitis vinifera*, AAF08329), VvSUC12 (AAF08330), VvSUC27 (AAF08331), ZmSUT1 (*Zea mays*, NP_001104840), ZmSUT2 (AAS91375), ZmSUT4 (AAT51689). Bars indicate the evolutionary distance.

Effect of auxin on *RhSUC2*, *3* and *4* transcript level in buds

RhSUC2, *3* and *4* transcript level in buds grown *in vitro* in the presence of MS media alone or supplemented with 0.1, 1 or 10 μM of synthetic auxin (NAA) for 72 h under light was estimated by quantitative RT-PCR. Dormant buds before treatment served as control (Fig. 7).

Compared to dormant buds, *RhSUC2* transcript level is higher in buds grown 72 h *in vitro* on MS media under light. This light-induced bud break and *RhSUC2* expression were reduced in MS medium supplemented with 1 and 10 μM of synthetic auxin (NAA). No difference was found between samples of MS and 0.1 μM NAA-supplemented MS. Under

the same experimental condition, transcript level of *RhSUC3* and *RhSUC4* was insensitive to auxin application.

DISCUSSION

Light-mediated bud break requires sugar influx into bud

In *Rosa* sp., light is critical for bud burst after apical dominance release through beheading (Mor & Halevy 1980; Mor *et al.* 1980; Girault *et al.* 2008). Light is perceived by the bud itself, and promotes organogenesis and the outgrowth of the foliar primordia (Girault *et al.* 2008). These processes are accompanied by a shift in sugar metabolism

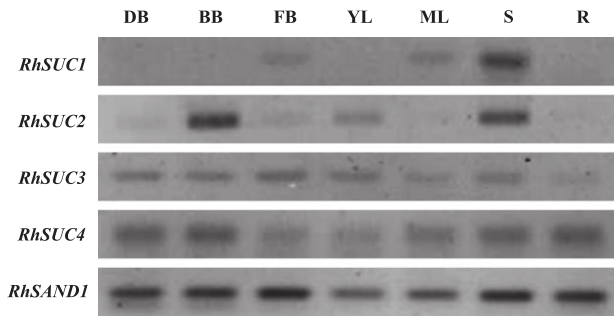


Figure 4. Transcript level of *Rosa hybrida* putative sucrose transporter genes in several organs. Total RNA was isolated from different tissues: dormant buds (DB), bursting buds (BB), flower buds (FB), young leaves (YL), mature leaves (ML), stems (S) and roots (R). Transcript levels were subsequently estimated by semi quantitative RT-PCR using gene-specific primers. *RhSAND1*, a stably expressed gene, was used as a control. Experiment was repeated twice.

(Girault *et al.* 2010). Light-induced bursting buds displayed a high import of sucrose and glucose, and an increase in transcript accumulation and enzymatic activity of vacuolar acid invertase (Girault *et al.* 2010). Taken together, these results suggest that bud outgrowth in the light is closely linked to the mobilization of sugars. This is in agreement with our findings, supporting a possible role of sucrose transport in the photocontrol of bud burst. Here, *in vitro* experiments show that buds require the import of exogenous metabolizable sugars (mainly sucrose) to develop under light (Fig. 1; Table 1). Similarly, an exogenous source of metabolizable sugars promotes *in vitro* development of palm bud (Al-Khateeb 2008). The optimal sugar concentration for *in vitro* bud development (250 mM in *Rosa*, Table 1) closely matches those found in the phloem sap of several species (Verscht, Tomos & Komor 2006; Nadwodnik & Lohaus 2008). Organogenesis remains constant during sugar starvation, while bud elongation is highly diminished (Table 1), suggesting that this latter could be the main sugar-dependant process.

Other evidence indicates that *Rosa* buds do not store enough sugars to promote their own development, and thus need to import soluble sugars. Growing buds for 3 d in the absence of sugar caused a drop in their sugar content. In contrast, buds grown in the presence of sugars develop well and show a similar metabolizable sugar content as the control, supporting their high metabolic activity. Such elevated metabolic activity in the outgrowing bud has been previously reported in other ligneous species such as peach tree and walnut (Marquat *et al.* 1999; Maurel *et al.* 2004a; Decourteix *et al.* 2008). Furthermore, we demonstrated that this import is an active, light- and energy-dependant sucrose absorption occurring during bud outgrowth (Table 2). These modifications are in accordance with an increase in plasmalemma ATPase activity (Aue *et al.* 1999; Alves *et al.* 2001) and active sugar absorption (Marquat *et al.* 1999; Maurel *et al.* 2004b) in the bud or neighbouring stem region. In walnut stem, the

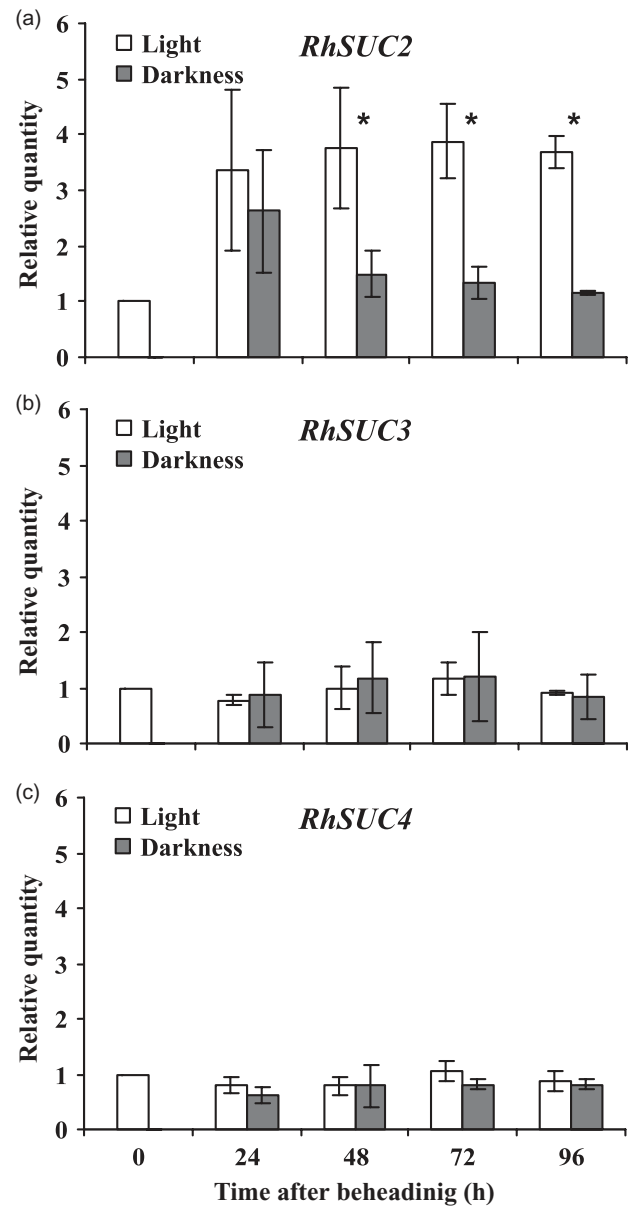


Figure 5. Transcript level of *Rosa hybrida* putative sucrose transporter genes in buds after beheading and growth under light or darkness. Plants at visible flower bud (VFB) stage were beheaded, their leaves were removed and plants were treated under light or darkness for 24–96 h. Untreated plants were used as control. *RhSUC2* (a), *RhSUC3* (b) and *RhSUC4* (c) transcript levels were estimated by real-time quantitative PCR using gene-specific primers. Gene transcript level is expressed relatively to control and normalized to *RhSAND1* transcript level. Each value is a mean of three independent biological replicates. The error bars represent standard errors. Asterisks indicate significant differences between light and darkness conditions (Student's *t*-test, $P < 0.05$).

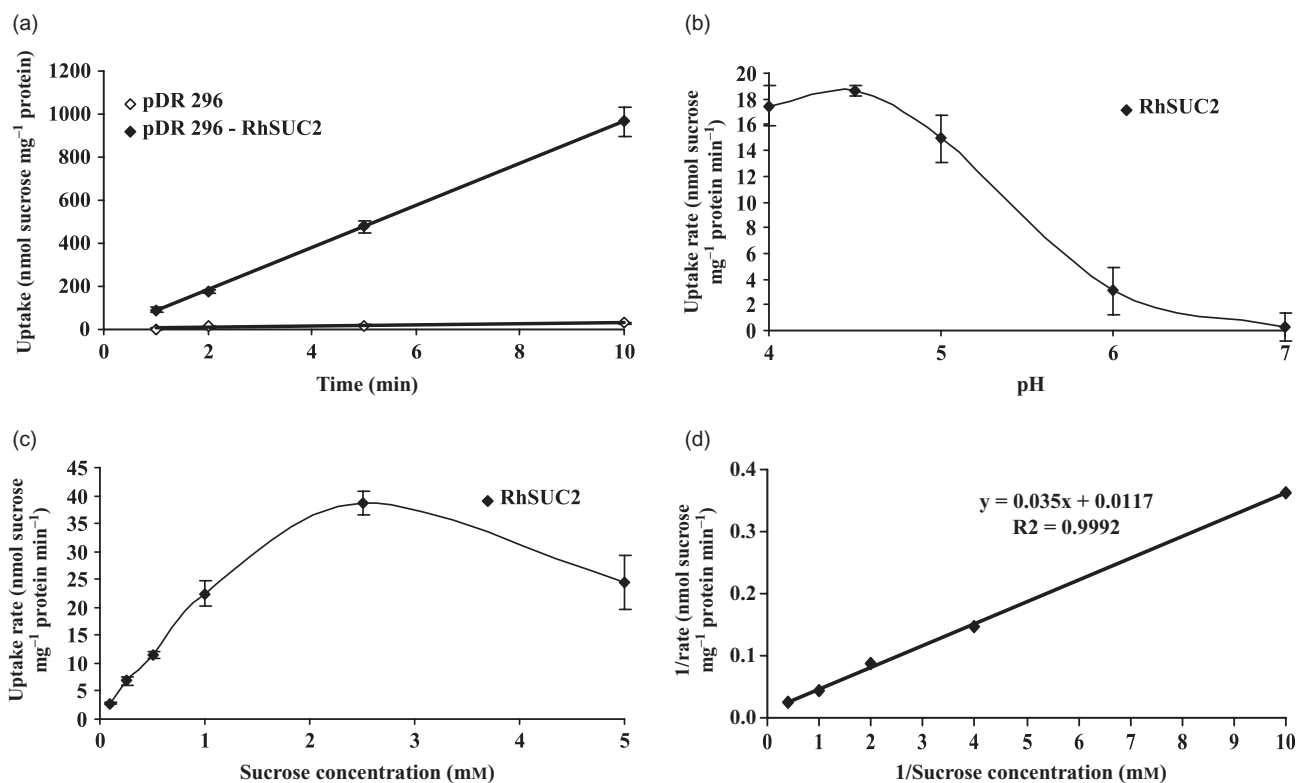


Figure 6. Characterization of the sucrose transport activity of RhSUC2 in baker's yeast *Saccharomyces cerevisiae* SUSY7. (a) Time course of sucrose uptake (pH 4.5, 0.5 mM sucrose). (b) pH dependence of sucrose uptake (0.5 mM sucrose). (c) Sucrose concentration-dependent sucrose uptake (pH 4.5). Bars represent standard errors ($n = 3$). (d) Lineweaver–Burk transformation of data presented in (c). Regression line is shown for data obtained at sucrose concentration below 2.5 mM. Kinetic parameters are $K_m = 2.99$ mM and $V_{max} = 21.41$ nmol sucrose mg⁻¹ protein min⁻¹.

expression of the putative sucrose transporter *JrSUT1* is correlated to a bud sink strength increase during outgrowth (Decourteix *et al.* 2008; Bonhomme *et al.* 2010). Our data clearly show that *Rosa* bud is a sink organ requiring importation of metabolizable sugars, at least sucrose, to ensure correct outgrowth in light.

Table 3. Effect of competing sugars and inhibitors on sucrose uptake by RhSUC2 expressed in baker's yeast SUSY7

Treatment	% Activity
Competing sugar	
Control	100
25 mM Sucrose	21 ± 6.6*
25 mM Glucose	140 ± 7.2*
25 mM Fructose	157 ± 4.4*
25 mM Raffinose	103 ± 3.9
25 mM Maltose	43 ± 2.2*
Inhibitors	
Control	100
50 μM CCCP	25 ± 2.5*
100 μM PCMBs	9 ± 2.0*

* represents a significant difference between the control and the treatment (Student's *t*-test, $P < 0.001$).

Rosa bud contains several sucrose transporter isoforms

We have identified four putative sucrose transporters (*RhSUC1*, *RhSUC2*, *RhSUC3* and *RhSUC4*) confirming that they are encoded by a small multigenic family. Depending on the genus, the number of isoforms is different: two in tobacco (Bürkle *et al.* 1998; Lemoine *et al.* 1999), three in tomato (Barker *et al.* 2000; Weise *et al.* 2000), four in grapevine (Davies *et al.* 1999; Ageorges *et al.* 2000), five in rice (Aoki *et al.* 2003; Hirose *et al.* 2010), seven in *Hevea* (Dusotoit-Coucaud *et al.* 2009; Tang *et al.* 2010) and nine in *Arabidopsis* (Sauer 2007 for review).

The isolated putative sucrose transporters share a high sequence identity with those from herbaceous and ligneous plants (Fig. 2), particularly RhSUC4 with apple tree (77%) and other isoforms with *Hevea* (66–77%). Furthermore, the four putative sucrose transporters are spread within three phylogenetic clades defined by Sauer (2007) that groups all known dicotyledonous sequences. Group 2 contains high affinity, low capacity transporters implicated in phloem loading and sucrose import in sink cells. RhSUC1 and RhSUC2 are homologs of these transporters. RhSUC3 is similar to group 3 transporters that contain low affinity and high capacity transporters found in sieve elements and in

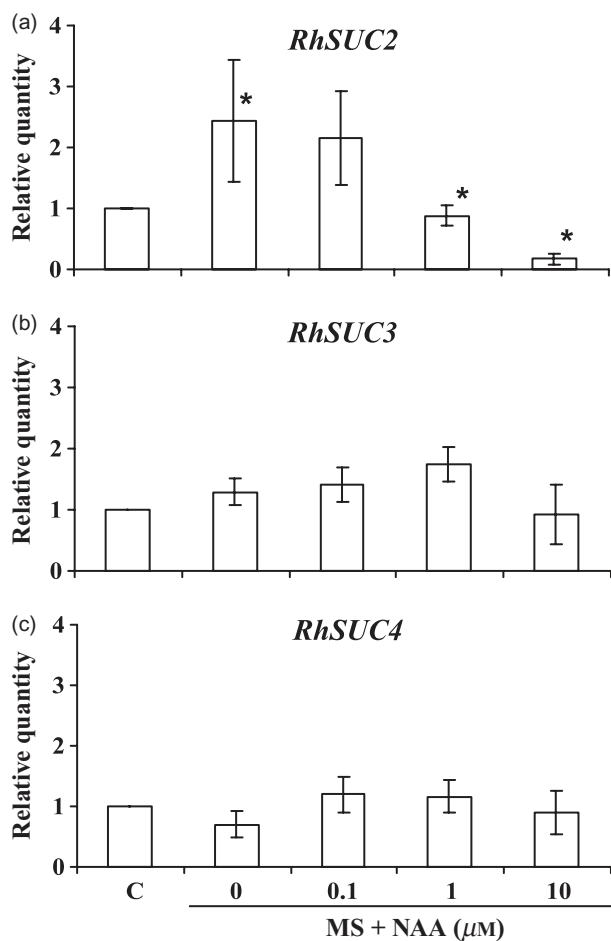


Figure 7. Effect of auxin treatments on *RhSUC2* transcript level in buds. Plants at visible flower bud (VFB) stage were used for treatments. Buds were grown *in vitro* in the presence of basic Murashige and Skoog media (MS) alone or added with 0.1, 1 or 10 μM of *N*(-1-naphthyl) acetic acid (NAA) (MS + NAA) during 72 h under light. Dormant buds before treatment served as control (C). Transcript levels were estimated by quantitative RT-PCR using gene-specific primers. Gene transcript level is expressed relatively to control and normalized to *RhSAND1* transcript level. Each value is a mean of three independent biological replicates. The error bars represent standard errors. Asterisks indicate significant differences with the control (Student's *t*-test, $P < 0.05$).

several sink tissues where they may mediate sucrose efflux during phloem unloading and act as sucrose sensors. *RhSUC4* belongs to group 4, containing tonoplast transporters mainly found in sink tissues (Sauer 2007).

RhSUC2 could be implied in bud sucrose loading during light-controlled bud burst

Many sucrose transporters are expressed in various sink tissues where they are implicated in several different physiological processes such as tuberization (Kühn *et al.* 2003), fruit formation (Davies *et al.* 1999; Ageorges *et al.* 2000; Hackel *et al.* 2006; Zhang *et al.* 2008), seed formation

(Weber *et al.* 1997; Baud *et al.* 2005; Hackel *et al.* 2006; Zhou *et al.* 2007), latex synthesis (Dusotoit-Coucaud *et al.* 2009, 2010; Tang *et al.* 2010) and pollen development (Lemoine *et al.* 1999; Stadler *et al.* 1999; Takeda *et al.* 2001). In *Rosa*, quantitative and semi-quantitative RT-PCR showed that three of the four putative sucrose transporters (*RhSUC2*, *RhSUC3* and *RhSUC4*) are expressed in the bud (Figs 3 & 4), with high accumulation of *RhSUC2* in bursting buds (Fig. 3). Furthermore, it is the only sucrose transporter whose expression was correlated with light-induced bud break in beheaded plants. This treatment induces the outgrowth of axillary buds in the presence of light but not in dark (Khayat & Zieslin 1982; Girault *et al.* 2008). Under light conditions, *RhSUC2* transcript level increases within 48 h and remains high during the entire 96 h time course relative to dormant bud (dark condition). In contrast, we found no correlation between the expression profiles of *RhSUC3* and 4, and bud outgrowth potential in the presence of light, reinforcing the potential role of *RhSUC2* in light-induced bud break. In light, the accumulation of *RhSUC2* transcript in the bud is concomitant with enzymatic vacuolar acid invertase activity (Girault *et al.* 2010), which is a major component of organ sink strength (Nägele *et al.* 2010) and cell elongation (Morris & Arthur 1984; Sturm 1999). Within this context, the imported sucrose can contribute to cellular growth processes, such as carbon skeleton and energy source, and also as osmotically active molecules required for cell expansion. Likewise, the sucrose imported into the bud or its cleavage products (hexoses) may be a signal molecule to regulate genes involved in development (see Gibson 2005 for review).

The transport activity of *RhSUC2* was tested in baker's yeast. We found that the heterologous expression of *RhSUC2* demonstrated that it encodes a functional, high affinity sucrose/H⁺ co-transporter. Its transport activity is sensitive to PCMBs (thiol inhibitor) as reported for several sucrose transporters (Sauer & Stolz 1994; Ludwig, Stolz & Sauer 2000; Meyer *et al.* 2000; Barth, Meyer & Sauer 2003) and quite specific to sucrose because only maltose appears as a competitor for sucrose transport (Table 3). Interestingly, the sucrose transport activity of *RhSUC2* was repressed by sucrose itself, at concentration (5 mM) close to the K_m value. Such regulation has already been observed in yeast, where the HXT2 hexose transporter expression is repressed by high level of glucose, at transcriptional and posttranslational levels (Wendell & Bisson 1994). Physiological significance of such regulation of *RhSUC2* deserves to be clarified in the near future.

Taken together, these results strongly suggest that *RhSUC2* can play a central role in sucrose influx into outgrowing buds. Sink organs actively import sugars such as sucrose, but also as hexose or polyols (Gao *et al.* 2003; Büttner 2007; Sauer 2007; Dusotoit-Coucaud *et al.* 2010). In vegetative buds of peach tree, physiological data indicated the existence of an active uptake of glucose that is concomitant with the stimulation of cell wall invertase (Maurel *et al.* 2004a), and such co-existence was reported in many sink organs (Sherson *et al.* 2003; Hayes, Davies & Dry 2007).

Potential involvement of hexose transporters in light-induced bud break in *Rosa* could not be ruled out, even if no cell wall invertase induction occurred concomitantly with bud burst (Girault *et al.* 2010).

Numerous *Rosaceae* transporters contain both sucrose and sorbitol transporters that play different roles in sink organs (Gao *et al.* 2003; Dusotoit-Coucaud *et al.* 2010). However, it does not seem to be the case in *Rosa*, because sorbitol was not detected in phloem sap, in stems or buds (Zimmermann & Ziegler 1975; Girault *et al.* 2010). Furthermore, exogenous sorbitol (100 mM) induces only a limited bud burst and prevents it at higher concentrations (Table 1). It seems therefore unlikely that polyol transporters are implicated in sugar importation during bud burst.

***RhSUC2* could be regulated by the combined effect of light and beheading**

Beheading transiently induces *RhSUC2* expression in buds after 24 h, independently from light (Fig. 5). This may be caused by a wound effect or to hormonal changes induced by apical dominance suppression. Previous work demonstrated that wounding (Meyer *et al.* 2004) and different hormones such as ethylene (Chincinska *et al.* 2008; Dusotoit-Coucaud *et al.* 2009, 2010; Tang *et al.* 2010), gibberelins (Chincinska *et al.* 2008) and cytokinins (Harms *et al.* 1994) all affect different kinds of sucrose transporters at the transcriptional level. In our system, beheading is used to suppress apical dominance, via a reduction of the auxin flux (Shimizu-Sato, Tanaka & Mori 2009). Auxin application (1 and 10 μ M) prevents stimulation of *RhSUC2* expression under light (Fig. 7), which was correlated with the absence of bud break *in vitro* (data not shown; Prasad *et al.* 1993). This finding suggests that the effect of light on *RhSUC2* expression was negatively affected by auxin and could occur in the absence of apical dominance.

The effect of light on *RhSUC2* expression is in agreement with previous work implying several sucrose transporters. In rice, *OsSUT1* mRNA accumulates in light, but is absent in dark (Matsukura *et al.* 2000). In carrot, the levels of *DcSUT1* transcripts were light dependent, with high levels during the day and low levels during the night (Shakya & Sturm 1998). In potato, *StSUT4*-RNAi transgenic plants resulted in an early flowering, high tuber production and a reduced sensitivity towards light enriched in far-red wavelengths – therefore mimicking shading (Chincinska *et al.* 2008). In potato model, Kühn & Grof (2010) have proposed that *StSUT4* expression is co-regulated by light via phytochrome B.

In conclusion, we show that photocontrol of bud burst is highly correlated with sugar import in buds. This process requires an active sucrose transport, probably mediated by *RhSUC2*. This finding supports a new function of sucrose transporter in plant physiology. Moreover, this work lays the foundation to deepen the molecular mechanisms by which light regulates *RhSUC2* expression and therefore to

better understand *RhSUC2* involvement in the modulation of plant shape by light condition.

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