Sugar availability is involved in rose bud outgrowth stimulation following a temporary light intensity restriction during the vegetative development of the main stem

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Abstract

Branching is a major agronomic variable determining yield and quality, and is very sensitive to environmental conditions. Previous studies on rose showed that a continuous high light intensity perceived during the bud outgrowth period stimulated bud outgrowth compared to a continuous low light intensity. This effect was related to higher cytokinin contents in the nodes. Interestingly, a temporary light intensity restriction applied before the bud outgrowth period over-stimulated bud outgrowth, but the mechanisms involved remain unknown. In this case, we assume a non-limitation in cytokinins because of the current high light intensity during the bud outgrowth period, but an increase in sugars that would explain bud outgrowth stimulation. To test sugar involvement, we quantified bud outgrowth, sugar contents, and the balance between sources and sinks for sugars in the bud outgrowth period of plants grown under either continuous high light intensity or under a temporary light restriction followed by a high light intensity. In addition, we quantified the effect of exogenous sugar supply on bud outgrowth for plants under continuous high light intensity, and the effect of leaf masking under the non-continuous treatment. Our results showed that after a temporary light intensity restriction and return to high light intensity, sugars accumulated compared to a continuous high light intensity. Furthermore, the growth of apical organs was reduced indicating that sugar accumulation might be due to a higher balance between sources and sinks for sugars. Exogenous sucrose supply through the petiole of intact plants grown under high light intensity stimulated bud outgrowth. Conversely, leaf masking after a temporary light intensity restriction inhibited bud outgrowth. This supports that sugar accumulation is an important trigger of bud outgrowth after a temporary light intensity restriction. Together these results indicate that an anterior low light intensity applied during the main stem development reduces growth of apical organs while higher sugar availability afterward favors lateral bud outgrowth.

Keywords: bud outgrowth, light intensity, branching, sugar, source-sink

INTRODUCTION

Plant branching is an important agronomic trait as it determines final yield (Whiting et al., 2005), and is involved in sanitary (Simon et al., 2012) and visual quality (Boumaza et al., 2010) of productions. Branching is highly responsive to environmental factors such as nitrogen fertilization, water supply, temperature, or light (Lafarge et al., 2010; Djennane et al., 2014; Furet et al., 2014; Li-Marchetti et al., 2015; Corot et al., 2017;). Thus, understanding and predicting branching response to environmental conditions is essential

to improve technical itineraries and culture ideotypes. We focus our study on the impact of light intensity on bud outgrowth, which determines early steps of branching.

Bud outgrowth is inhibited by low (versus high) light intensity imposed during bud outgrowth period, as observed for rose (Roman et al., 2016; Corot et al., 2017). This inhibition was correlated to low cytokinin biosynthesis and level, and to low sugar level in the stem. However, only cytokinins were shown to be involved in bud outgrowth regulation in response to low light intensity, since exogenous sugar supplies did not restore any bud growth under this non-permissive light condition (Roman et al., 2016; Corot et al., 2017).

Bud outgrowth is also sensitive to anterior light intensity. Demotes-Mainard et al. (2013) reported for rose an overstimulation of bud outgrowth under high light intensity after a temporary restriction of light intensity (LH treatment), compared to continuous high light intensity (HH treatment). The limiting role of cytokinins, demonstrated for low light intensity during bud outgrowth, is unlikely true in this case, since plants under both light treatments are under high light intensity during bud outgrowth period. We will thus look for the possible role of sugars in bud outgrowth stimulation under LH treatment. Under this treatment, apical leaves and internodes of the primary stem, which are still growing during bud outgrowth period, are initiated under low light intensity. Granier and Tardieu (1999) demonstrated for sunflower that low light intensity during leaf initiation reduced leaf elongation rate persistently, even after restoration of high light intensity. This indicates that growing organs of the primary stem may represent lower sugar sinks during bud outgrowth period under LH treatment compared to HH, and that subsequent primary axis competition for sugars is reduced under LH treatment. Recent studies also support a possible role of sugars, since their signal role in bud outgrowth stimulation has been demonstrated for rose and pea (Mason et al., 2014; Barbier et al., 2015)

The objective of this paper is to determine whether sugars are involved in bud outgrowth stimulation after a temporary light intensity restriction (LH treatment), and whether this can result from primary axis growth reduction, leading to an increase of available sugars for bud growth.

MATERIALS AND METHODS

Plant material and growth conditions

Plants were obtained from cuttings of *Rosa hybrida* 'Radrazz' as described in Demotes-Mainard et al. (2013). Well-rooted cuttings were grown in 500 ml pots containing a 50/40/10 mixture of neutral peat, coconut fibers, and perlite. After a short growth in a heated greenhouse (until three leaves were visible), plants were transferred to a growth chamber (light/dark 16/8h photoperiod; 22/20°C at day/night; humidity was maintained between 60 and 70%). Water and mineral nutrition (5,0 mM KNO_3, 2,0 mM $Ca(NO_3)_2$, 2,0 mM NH_4NO_3, 2,0 mM KH_2PO_4, 2,0 mM $MgSO_4$, 0,25 mM NaOH) were provided by sub-irrigation to maintain the plants in comfortable water and mineral conditions.

Light treatments

Plants in growth chamber were submitted to two different regimes of light intensity in the Photosynthetically Active Radiation (PAR) : (1) a continuous high PAR intensity (300-320 µmol/m^2/s) (referred to as HH), (2) a low PAR intensity (80-100 µmol/m^2/s) until the appearance of the flower bud (FBV : flower bud visible on the main stem), and a high PAR intensity from FBV onwards (treatment LH).

Morphological measurements

For all experiments, the state (dormant or outgrown) and length of each bud were monitored three to four times a week since FBV + 4 days. A bud was considered has grown out when the first leaf was clearly visible above the bud scales (Girault et al., 2008; Corot et al., 2017). As soon as the third leaf of the primary axis appeared, length of the final leaflet of each leaf of the primary axis was measured every two days until wilted flower bud stage. At

wilted flower stage, leaves were excised and scanned. Images were treated using ImageJ software to estimate final area of each leaf of the primary axis.

Photosynthesis measurements

Under both light treatments, CO_2 net assimilation rate per leaf surface unit per second was performed 4 times between FBV and FBV + 7 days on the second most basal leaf on the primary axis on entire plants, at a temperature of 20°C, and an ambient CO_2 concentration of 400 mmol mol^{-1} (Li-Cor Inc., Lincoln, NE, United States).

Quantification of endogenous sugars

Roots, stem, leaves and flower button of the primary axis were collected at 7 days after FBV stage on entire plants grown under LH and HH regimes. Sampling were started 3h after the beginning of the light period, frozen in liquid nitrogen, and stored at -80°C before lyophilization and grounding. Sucrose and starch were determined by colorimetry.

Manipulating plant sugar contents

To avoid any interaction with the apical part of the plant, which is a strong sink for sugars, experiments of sugar manipulation were undertaken on plants decapitated at FBV. These plants were obtained by removing all the plant parts 2 cm above the fourth node bearing a true leaf (counting from plant base). Regarding the remaining leaves, all leaflets except a most basal one, were removed to limit photosynthetic sugar content. To maintain auxin-mediated apical dominance, a 2 ml-tube containing a basic medium (1% agar, 2,5 $ml.l^{-1}$ PPM), supplemented with a synthetic auxin (10µM 1-naphthaleneacetic acid, NAA), was applied at the cut end of the decapitated stump.

Decapitated plants grown under HH treatment were supplied with sucrose (25 or 50 mM) or mannitol (50 mM), an osmotic control, through the 4th petiole as described in Lin *et al.*, (2011). The petiole was rapidly immersed in a sugar-containing liquid solution in a 1.5 ml reservoir. After 1 week the petiole was cut 0,5 cm lower.

The four leaflets of decapitated plants grown under LH treatment were half covered with black plastic sheets or transparent plastic (control).

Statistical analysis

Statistical analyses were performed using R software (R Core Team, 2014). Groups were compared using Student's or Fisher's test. Significant differences (p-value <0.05) are indicated with the symbol *.

RESULTS

A temporary restriction in PAR intensity before bud outgrowth stimulated bud outgrowth and plant sugar status

In accordance with previous results of Demotes-Mainard et al. (2013), a temporary period of low PAR intensity (treatment LH) stimulated significantly bud outgrowth on rose primary axis, compared to continuous high PAR intensity (treatment HH). The total percentage of axillary buds that grew out was 12 % higher for LH than for HH treatment 10 days after the flower bud was visible on the primary axis (FBV stage) (Table 1). We showed that plant starch content was also multiplied by 1.64 for LH compared to HH at FBV + 8 days, while stem sucrose contents was similar between both treatments. This demonstrates an excess of sugars in LH compared to HH, and indicates that this excess may explain in part the stimulation of bud outgrowth in LH compared to HH.

Sugar stimulates bud outgrowth under high light intensity

To test whether the sugars excess in LH compared to HH is involved in bud outgrowth stimulation, we either (i) brought exogenous sucrose to plants in HH through petiole, or (ii) masked leaves of plants in LH to decrease sugar supply by photosynthesis. In HH, exogenous sucrose supply increased by 25% total bud outgrowth frequency compared to the osmotic

control (figure 1A). Conversely, in LH, masking leaves reduced by almost 70% bud outgrowth percentage (figure 1B). Thus, this indicates that sugar excess in LH has a role in bud outgrowth stimulation.

The sources/sinks balance of sugars is higher under LH treatment compared to HH treatment

To understand the sugar excess under LH compared to HH, we estimated the source and sink strengths for sugars for both treatments. At FBV, just before the onset of bud outgrowth, the four most basal phytomers had finished their elongation, whereas upper phytomers were still elongated (data not shown). We therefore considered the four most basal leaves as the main sources of sugar, and the upper organs as the main sinks of sugars. The photosynthetic area of the main source leaves was similar in HH and LH (118 cm^2) at FBV, and photosynthesis per unit area was 26% lower for LH compared to HH (Table 2) during 7 days after FBV. Thus, source strength of sugars was lower in LH than in HH and does not explain the excess of sugars observed in LH compared to HH. The final mass of the main sinks was 39% lower in LH compared to HH, indicating a lower sink strength for sugars in LH compared to HH. Sugar excess in LH condition may be thus explained by a favorable sources-sinks ratio due to lower growth of the upper organs during the period of bud outgrowth.

Table 1. Effect of light treatments on intact plant bud outgrowth frequency at FBV +10 days, and on total plant sugars content at FBV + 8 days. Plant bud outgrowth frequencies are means of at least 14 plants per treatment ± SE. Plant sucrose and starch content are means of at least 3 plants per treatment ± SE.

Bud outgrowth and sugars contents	**LH**			**HH**			
% of outgrown buds per plants at FBV + 10d	48,9	±	2,3	36,9	±	2,0	*
Plant sucrose content (µmol gluc/gDW)	527,7	±	12,9	537,2	±	31,7	
Plant starch content (µmol gluc/gDW)	527,7	±	3,1	58,2	±	4,1	*

Table 2. Effect of light treatments on sources and sinks of sugars. (i)Surfacic photosynthetic capacity during 7 days after FBV. Values are means ± SE of 4 plants measured at 4 dates between FBV and FBV +7 days . (ii)Total surface of photosynthetic leaves at FBV (leaves 1 to 4 from the base of the plant). Values are means of at least 14 plants per treatment ± SE. (iii) Final dry mass of still growing apical organs after FBV (leaves and internodes upper the fifth phytomer). Values are means of at least 14 plants per treatment ± SE.

Sources and sinks of sugars	**LH**			**HH**			
Surfacic photosynthetic capacity (µmol/m^2/s)	8,4	±	0,5	11,3	±	0,3	
Total surface of photosynthetic leaves at FBV (cm^2)	118,4	±	7,2	118,7	±	5,4	
Total dry mass of aerial growing organs on the primary axis after FBV (g)	1,4	±	0,1	2,3	±	0,2	*

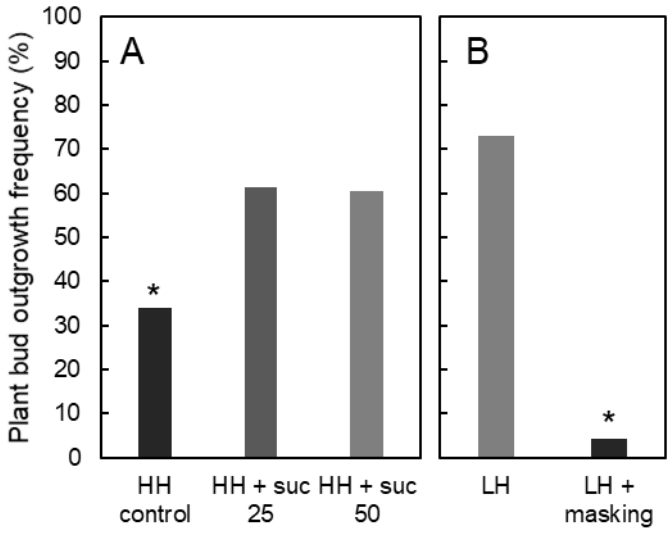

Figure 1. Effect of global sugar content manipulation under HH and LH treatments on total bud outgrowth frequency. For both experiments, plants were decapitated at FBV above the fourth leaf, and NAA agar (10µM) was applied on the top of the stem. A/ Effect of exogenous input of sucrose (25 and 50mM) under HH treatment compared to mannitol control (50mM). At least, 11 plants per treatment. B/ Effect of masking leaves under LH treatment. n = 12 plants per treatment.

DISCUSSION

Unfavorable environmental conditions are known to modulate bud outgrowth via hormonal and nutrient regulators (Roman et al., 2016; Corot et al., 2017). We aimed to validate the impact of a temporal unfavorable light intensity, applied before the branching period, on bud outgrowth, and to determine the role of sugars in this regulation. As observed previously by Demotes-Mainard et al. (2013), rose bud outgrowth frequency was significantly increased by a temporary light intensity restriction applied before the branching period (table 1), compared to a continuous high light intensity. Interestingly, bud outgrowth stimulation following a temporary light intensity restriction was correlated to sugar accumulation as starch, compared to the continuous high light treatment (table 1).

Because sugars are known to stimulate bud outgrowth (Mason et al., 2014; Barbier et al., 2015), we tested if sugars might be involved in the phenotype observed under high light intensity following a temporary light intensity restriction (LH treatment). To do so, we manipulated sugar supply in plants under the two light treatments (figure 1). The results support that sugars are involved in bud outgrowth stimulation under high light intensity after a temporary light intensity restriction.

Quantification of sugar sources and sinks revealed that starch accumulation may be due to lower sugar demand of the growing organs of the main axis following a restriction in light intensity (table 2). Such sugar accumulation after the recovery of comfortable conditions was previously observed for rice after a temporary shading during the development of the main stem (Lafarge et al., 2010).

Therefore, bud outgrowth stimulation by a temporary light intensity restriction might result of a lesser competition for sugars between main axis and lateral buds compared to plants grown under continuous high light intensity. Such modulation of bud outgrowth by the growth of the main axis was previously observed for wheat plants of different internode lengths (Kebrom et al., 2012).

A branching stimulation for rose was similarly observed after a temporary water restriction before the branching period compared to continuous comfortable hydric conditions (Demotes-Mainard et al., 2013), questioning the possibility of common

mechanisms of bud outgrowth stimulation by temporary unfavorable environmental conditions before the branching period.

CONCLUSION

A temporary light intensity restriction applied before the bud outgrowth period, during the development of the main axis, leads to a bud outgrowth stimulation. Our results indicate that smaller apical sinks of sugars after the high light intensity recovery can explain this phenotype.

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