

Sugar and Light Effects on the Condition of the Photosynthetic Apparatus of *Arabidopsis thaliana* Cultured in vitro

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Abstract Light and sugars are fundamental elements of plant metabolism and play signaling roles in many processes. They are also critical factors determining the condition of plants cultured in vitro. The aim of this work was to investigate the simultaneous influence of irradiance and sugar content in the medium on the growth and photosynthetic apparatus condition of *Arabidopsis thaliana* in vitro. Plants were grown on media containing 1 or 3% of sucrose or glucose at three irradiances: 25, 100, and 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (weak, medium, and strong light). Media without sugar were used for control plants. Plant growth parameters were measured and the following physiological processes were investigated: photosynthesis, blue light-induced chloroplast relocations, and xanthophyll cycle activity. The expression of genes related to these processes was analyzed. The presence of sugar in the medium was found to be essential for the growth of *Arabidopsis* in vitro. Weak light significantly limited growth and the capacity to acclimate to changing light conditions. Strong light was a source of stress in some cases. Contrary to earlier reports, exogenous sugars showed a positive effect on photosynthesis. At higher concentration they acted as photoprotectants, overcoming the negative influence of strong light on photosynthesis and the xanthophyll cycle. The expression of all investigated genes was influenced by irradiance and sugar presence. In many cases differential effects of sugar type and concentration could be observed. The specific effects of some irradiance/sugar

concentration combinations point to possible interactions between sugar- and light-induced signaling pathways.

Keywords *Arabidopsis thaliana* · Light · Sugars · Glucose · Sucrose · In vitro culture · Photosynthesis · Chloroplast movements · Xanthophyll cycle

Introduction

Both light and sugars play fundamental roles in plant growth and development. They are not only basic elements of plant metabolism, they also have other important functions. Light is essential for plants due to its role in photosynthesis. Apart from being the primary source of energy, light also plays a signaling and regulatory role in developmental and metabolic processes. This is possible thanks to photoreceptors that receive and transmit light signals (for reviews see Gyula and others 2003; Chory 2010). Among them are red and far-red light receptors (phytochromes), as well as blue light receptors (phototropins and cryptochromes). Light controls several developmental processes, for example, the induction of flowering (for review see Franklin and others 2005). In *Arabidopsis thaliana* light can induce flowering in two different ways: through the photoperiodic pathway or the light-quality perception pathway. In the first case, flowering is dependent on the length of day and night. The photoperiod is sensed by the plant thanks to phytochromes and cryptochromes that transmit light signals to the circadian clock. In favorable conditions the circadian clock activates a gene expression cascade leading to floral induction (Jaeger and others 2006). The second pathway is activated by changes in the light spectrum which are sensed by phytochrome B. Flowering is induced when the ratio of red to far-red light

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decreases. This change in light quality usually occurs in the shade. The induction of flowering under these conditions is part of the shade avoidance syndrome (Cerdán and Chory 2003). Excess light can, however, be a source of stress and damage the photosynthetic apparatus resulting in photoinhibition (for review, see Vass and others 2007). To prevent light stress, plants have developed several protective mechanisms that can limit light absorption, such as leaf blade movement, chloroplast movement, or the presence of leaf hairs. The excess of absorbed energy can be dissipated in the xanthophyll cycle (for review, see Demmig-Adams and others 1996).

Sugars play a central role in plant metabolism. Produced during photosynthesis, they are substrates for energetic processes and for the synthesis of metabolically and structurally important compounds such as amino acids, fatty acids, and cellulose. They are also the main storage material in plants. Sugars play a regulatory role in photosynthesis, growth, and development. This is not only the effect of metabolic changes, sugars can also act as signaling molecules. They can modulate gene expression and influence various signaling pathways. One of the sugar sensors in plant cells is hexokinase (Xiao and others 2000; Granot 2008). This enzyme phosphorylates hexoses (in vivo mainly glucose), which are later used in several important metabolic pathways, including glycolysis. The sensory and catalytic functions of hexokinase are independent and two separate signal transduction pathways can be distinguished: glycolysis-dependent and glycolysis-independent. Free hexoses that function as signaling molecules are not the direct product of photosynthesis; they are the product of poly- and oligosaccharide degradation. Sucrose is the main transportable sugar in plants and can be degraded by two enzymes: sucrose synthase and invertase (Koch 2004). The products are fructose and, respectively, UDP-glucose or glucose. This means that different amounts of free hexoses, which can act as signaling molecules, are produced depending on the enzyme. Sucrose itself can also induce specific signal transduction pathways (Chiou and Bush 1998; Wind and others 2010). Sucrose transporters or similar proteins probably act as sucrose sensors (Barker and others 2000). Sugars modulate the expression of numerous genes. Depending on the response to sugars, genes can be classified as “feast” or “famine” genes (Koch 1996; Rolland and others 2002). The former are upregulated by sugars and repressed in conditions of sugar depletion, and the latter are repressed in conditions of sugar abundance and upregulated by sugar deficiency. Among “famine” genes are those connected with photosynthesis and degradation of storage material: polysaccharides, lipids, and proteins. Their upregulation allows for an increase in the production of energetic substrates when they are deficient. When carbohydrate resources are

abundant, “feast” genes can be activated. They are connected with processes of sugar utilization (respiration) and storage. Sugar availability also enhances the expression of genes connected with defense mechanisms and secondary metabolite synthesis. Moreover, sugar levels regulate several developmental processes from embryogenesis to senescence (Rolland and others 2002; Gibson 2005). Exogenous sugars present in the medium can have a strong influence on plant development. One of the best known examples is the inhibition of germination and seedling growth by glucose (Dekkers and others 2004).

In vitro culture conditions differ significantly from the natural environment. This can be a source of stress for the plants. Most in vitro cultures are characterized by poor gas exchange, increased humidity, low irradiance, and the presence of sugars in the medium. These factors can have a strong influence on plant morphology and physiology. In these conditions light and especially sugars play specific roles. Low irradiance in in vitro cultures is often due to technical constraints. However, even when an increase in illumination is possible, it can have a negative effect and cause photoinhibition. Gas exchange in culture vessels is limited resulting in a low CO₂ level. These two factors inhibit autotrophic growth; therefore, plants must be provided with an additional carbon source, usually in the form of sugar in the medium. The presence of exogenous sugars is thought to limit photosynthesis and prevent the proper development of the photosynthetic apparatus. This has been proven by several experimental works (Hdider and Desjardins 1994; Van Huylenbroeck and Debergh 1996; Rycbczyński and others 2007) and is consistent with Koch’s (1996) theory on the inhibitory influence of sugars on photosynthesis. The photosynthetic activity of in vitro-grown plants may be important for successful acclimatization to greenhouse or field conditions (Lees and others 1991). The responses of different plants to in vitro culture conditions can, however, vary significantly. For example, exogenous sucrose had opposite effects on the photosynthetic activity of two species of grapevine (Galzy and Compan 1992) and two cultivars of rose (Langford and Wainwright 1987). Moreover, the sugar effect can be concentration dependent. In tobacco, 3% sucrose present in the medium enhanced photosynthetic activity and prevented photoinhibition, but a 5% concentration caused a decrease in photosynthesis (Tichá and others 1998; Kadleček and others 2003).

Plants grown in vitro are used for many different experiments, but the modulating effects of culture conditions (especially the presence of sugars) on the investigated phenomenon are often neglected. In this work we studied the simultaneous influence of sugar concentration and irradiance on the growth of *Arabidopsis thaliana* in vitro and the functioning of its photosynthetic apparatus. Our

aim was not only to find the optimal culture conditions but also to examine the potential links between light and sugar signaling pathways. Therefore, apart from growth parameters we also investigated physiological processes such as photosynthesis, chloroplast movements, and xanthophyll cycle. The expression of genes connected with these processes was analyzed.

Materials and Methods

Plant Culture

Wild-type *Arabidopsis thaliana* plants (ecotype Columbia) were grown in vitro from seed on agar-solidified Gamborg B5 media (40 ml per jar) containing 1 or 3% of sugar (sucrose or glucose). The medium pH was adjusted before autoclaving to 5.7–5.8. Because glucose inhibits germination, seeds germinated and seedlings were grown on media with 1% sucrose for 2 weeks before transfer to media with glucose. Two types of plants were used as controls: plants germinated and grown on media without sugar (control 1) and plants germinated on media with 1% sucrose and transferred to media without sugar after 2 weeks (control 2). For the first 3 weeks plants were cultured in a growth chamber (Sanyo) at $21 \pm 1^\circ\text{C}$, a 10-h light/14-h dark photoperiod, under fluorescent lamps (Sanyo FL40SS W/37 and Osram L36 W/77). The average photosynthetic photon flux density at plant level was $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (medium light, ML). This is the standard irradiance used in growth chambers. Subsequently, part of the plants were transferred to a different growth chamber and cultured at $25 \pm 2^\circ\text{C}$, a 10-h light/14-h dark photoperiod, under a LED panel (ELEX-5AA0-D00 warm white LEDs, Edison Opto Corporation, Taiwan). The plants were cultured at two irradiances differing by one order of magnitude. The irradiances at plant level were on average $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (strong light, SL) and $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ (weak light, WL). The remaining plants were continuously cultured in ML. Experiments were performed on plants aged 5–6 weeks.

Growth Parameter Measurements

Leaf number and total leaf area per plant were determined at the age of 5 weeks. Leaves were detached from the plant and photographed and leaf area was calculated using Image-Pro Plus software.

Chlorophyll Fluorescence Measurements—Light Curves

Chlorophyll *a* fluorescence emission from the upper surface of leaves was measured with a pulse amplitude

modulation fluorometer (PAM210, Walz). Plants were dark-adapted for 20 min prior to measurements, then leaves were detached and used in the experiment. First, the maximum quantum efficiency of PSII (F_v/F_m) was measured in dark-adapted plants. This measurement was followed by 12 steps of illumination with actinic (photosynthetically active) light of increasing intensity. Each illumination step lasted 2 min and was followed by a measurement of the effective quantum yield of PSII ($\Phi_{\text{PSII}} = (F'_m - F_t)/F'_m$). The following actinic irradiances were used: 5, 55, 85, 115, 145, 205, 305, 435, 595, 845, 1,245, and 1,855 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Light curves were recorded using PamWin software. The program calculates electron transport rates (ETR) on the basis of Φ_{PSII} measurements according to the equation: $\text{ETR} = \Phi_{\text{PSII}} \times \text{PAR} \times 0.5$, where PAR is the actinic irradiance and 0.5 is the multiplication factor which results from the fact that the transport of one electron requires the absorption of two quanta of light. Light curves are plots of ETRs versus actinic irradiance.

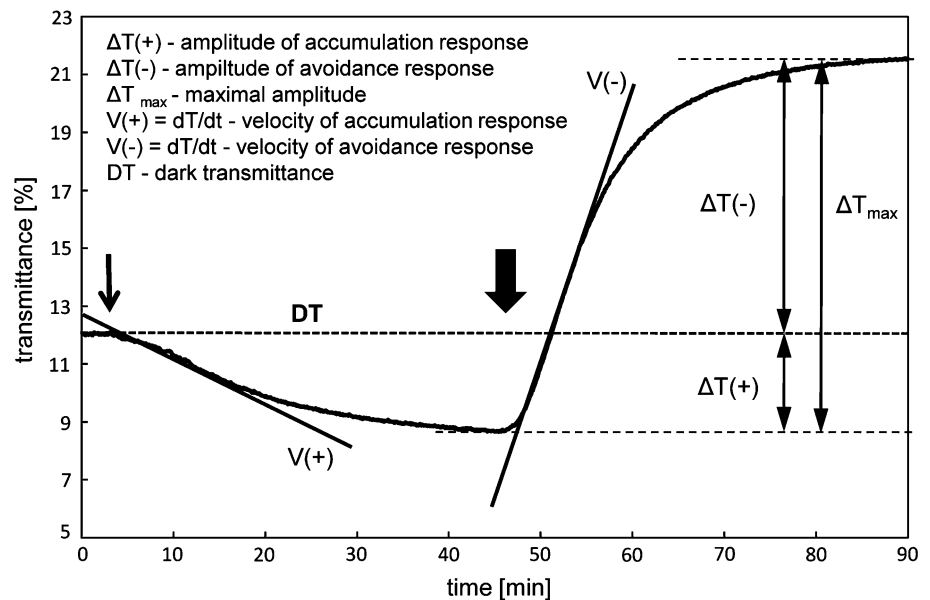
Chloroplast Movement Measurements—Photometric Method

Quantitative measurements of chloroplast movements were performed using a double-beam photometer (Walczak and Gabryś 1980). It records changes in the transmittance of measuring light (red light, 660 nm, $0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$, modulated with a frequency of 800 Hz) through the leaf. These transmittance changes are caused by chloroplast redistribution in the cells induced by blue light. Plants were dark-adapted for at least 16 h before the experiment. Leaves with similar transmittance levels after dark adaptation were selected for further measurements. After recording the initial level of transmittance, the leaves were illuminated with weak blue light ($1.6 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 45 min and then with strong blue light ($120 \mu\text{mol m}^{-2} \text{s}^{-1}$) for the same period. The following parameters were measured/calculated for each response: (1) amplitude—transmittance change after 45 min, and (2) velocity—first derivative of the initial linear fragment of the transmittance curve. Calculations for these parameters are shown in Fig. 1.

Xanthophyll Cycle Pigment Analysis—HPLC

To induce the operation of the xanthophyll cycle, plants were subjected to the following treatment: (1) dark adaptation for 16 h, (2) illumination with strong white light ($1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 20 min, (3) dark adaptation for 24 h. After each of these steps a sample was taken from the plant (2–3 leaves) and immediately frozen in liquid nitrogen. Pigments were extracted from the samples by grinding the leaves in a mortar with Solvent A (acetonitrile:methanol:water 72:8:1) and a small amount of CaCO_3 . The

Fig. 1 A model photometer recording trace showing the way in which amplitudes and velocities are calculated. Arrows indicate consecutively the onset of weak ($1.6 \mu\text{mol m}^{-2} \text{s}^{-1}$) and strong ($120 \mu\text{mol m}^{-2} \text{s}^{-1}$) blue light illumination



suspension was centrifuged and the supernatant analyzed by RP-HPLC on a Nucleosil 100 C-18 column (5- μm particle size, Teknokroma) in Solvent A at a flow rate of 1.5 ml/min. The eluted pigments were monitored at 440 nm. Chromatograms were analyzed using JASCO ChromPass software and the percentage of xanthophyll cycle pigments was calculated.

RNA Isolation, RT-PCR, and Semiquantitative PCR

Total RNA was isolated from leaves of 6-week-old plants using Trizol reagent. First-strand cDNA synthesis was performed using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas) with 500 ng of total RNA and random hexamer primers. cDNA was used as a template for PCR amplification with the following gene-specific primers:

UGP1 (forward: 5'-AAGCTCGATACTTTCTTATCACAG G-3', reverse: 5'-CGAGCTCGACTATACTAGGAATGG-3')

CABs (forward: 5'-AAGTACTTGGGTCCATTCTCTGG-3', reverse: 5'-GAATCCAAACATAGAGAACATAGC-3')

PHOT1 (forward: 5'-CATCGCAGAGAACTCGCAAAC-3', reverse: 5'-AGATTTCGTCGATTTCGCTTAGTGC-3')

PHOT2 (forward: 5'-GACGCTACACAGCCTCACTGT CCC-3', reverse: 5'-TCCCAACTGTCCCTCTGCCCT ATT-3')

VDE1 (forward: 5'-AACTTGTGCATGCTTATTGAAG G-3', reverse: 5'-AACAAGGTCATCTCAGTCCTACC-3')

ABA1 (forward: 5'-TCCTGGTTTTACTTGGGGTAAAG G-3', reverse: 5'-TCGAAGATCCATCAAGAAGAAA GC-3')

The *CABs* primers are specific to several members of the *CAB* gene family. QuantumRNA™ 18S RNA (Ambion)

with a 5:5 primer:competimer ratio was used as a standard. PCR products were separated in a 2% agarose gel containing 0.5 $\mu\text{g/L}$ of ethidium bromide.

Results and Discussion

Plant Growth and Development

Different culture conditions (irradiance and sugar concentration) resulted in varied plant phenotypes. All control plants, grown on media without sugar, were very small and their development was delayed, never reaching the generative phase. Most of these plants showed signs of vitrification. Plants grew much better in the presence of sugar in the medium. The kind of sugar (sucrose or glucose) and its concentration (1 or 3%) did not seem to have a significant effect. However, plants developed slightly different phenotypes depending on the irradiance. Plants grown in WL had smaller leaves, with long petioles extended in the direction of the light. Plants cultured in both ML and SL were well developed and reached the biggest dimensions. Vitrification appeared occasionally, usually in glucose-grown plants.

To confirm these observations the following growth parameters were measured: number of leaves and total leaf area. All control plants had a significantly smaller number of leaves than plants grown on sugar-supplied media (Fig. 2a). Among the latter no distinct differences could be seen. Only WL plants had a slightly lower leaf count. Total leaf area was also the lowest in control plants (Fig. 2b). For plants grown on 1% sugar, total leaf area was similar for ML and SL plants and slightly lower for WL plants. For

plants grown on 3% sugar, leaf area increased with growing light intensity. This effect was especially visible in the case of glucose, with 3% glucose SL plants reaching the highest value.

The results of observations and growth parameter measurements clearly show that the presence of sugar in the medium is essential for the normal growth of *Arabidopsis* in vitro. The growth and development of control plants, cultured on media without sugar, were strongly impaired. Exogenous sugar supply is necessary throughout the plant's life, as can be seen on the example of control 2 plants. These plants were germinated and grown for 2 weeks on a 1% sucrose medium. However, after transfer to medium without sugar they did not grow better than plants cultured without sugar from the beginning (control 1). A 1% concentration of sugar in the medium seems to be sufficient for normal growth, and in most cases the type of sugar (sucrose or glucose) is not significant. Both ML and SL are appropriate for *Arabidopsis* culture in vitro, whereas WL limits

plant growth. However, some combinations of sugar and light may turn out to be especially advantageous for the plant in a certain aspect, as in the case of the exceptionally good growth of 3% glucose SL plants. Increasing sugar concentration has a visibly stronger influence on leaf area than on leaf number. This may indicate that sugar present in the medium is used mainly as a carbon source for biomass production and does not play a major signaling role in development.

Photosynthetic Status

The ETR parameter, calculated on the basis of chlorophyll fluorescence measurements, gives information about the overall efficiency of photosynthesis. Light curves, which are plots of ETRs versus actinic irradiance values, reflect the plant's capacity to acclimate to different light conditions. When photosynthesis is limited by the capacity of the electron transport chain, the maximum ETR (ETR_{max}) is achieved. At still higher irradiances, ETR values tend to decline, which is a result of photoinhibition. As PSII is very susceptible to light-induced damage, changes in ETR values are one of the first signs of stress.

As can be seen in Fig. 3a–c, plants grown in WL had the lowest photosynthetic efficiency. They achieved ETR_{max} at lower actinic irradiance values than ML and SL plants. Among plants cultured in ML, sugar content in the medium did not have a big influence on photosynthesis. Plants cultured in SL responded differently to increasing actinic irradiance depending on the kind and concentration of sugar present in the medium. Higher sugar concentration resulted in better photosynthesis, with glucose having a slightly stronger effect than sucrose.

The same experimental results are presented in a different configuration in Fig. 3d–f to highlight another aspect of the relationship between culture conditions and photosynthesis. At each sugar concentration, the following pattern could be observed: WL plants had the lowest photosynthetic capacity, ML plants the highest. This was most obvious in the case of control plants. At 1% sugar the difference between ETR values of SL and ML plants was diminished and at 3% sugar these values became equal. The differential effect of sucrose and glucose could be seen mostly in WL and SL.

The above results show that both irradiance and sugar content in the medium have an influence on the photosynthetic efficiency of plants grown in vitro. Plants grown in WL exhibit a low capacity to acclimate to increasing irradiation and easily undergo photoinhibition. In most cases, plants grown in SL also have a limited photosynthetic efficiency compared to ML plants. This means that SL can be a source of stress for the plants, impairing the development of the photosynthetic apparatus in some way.

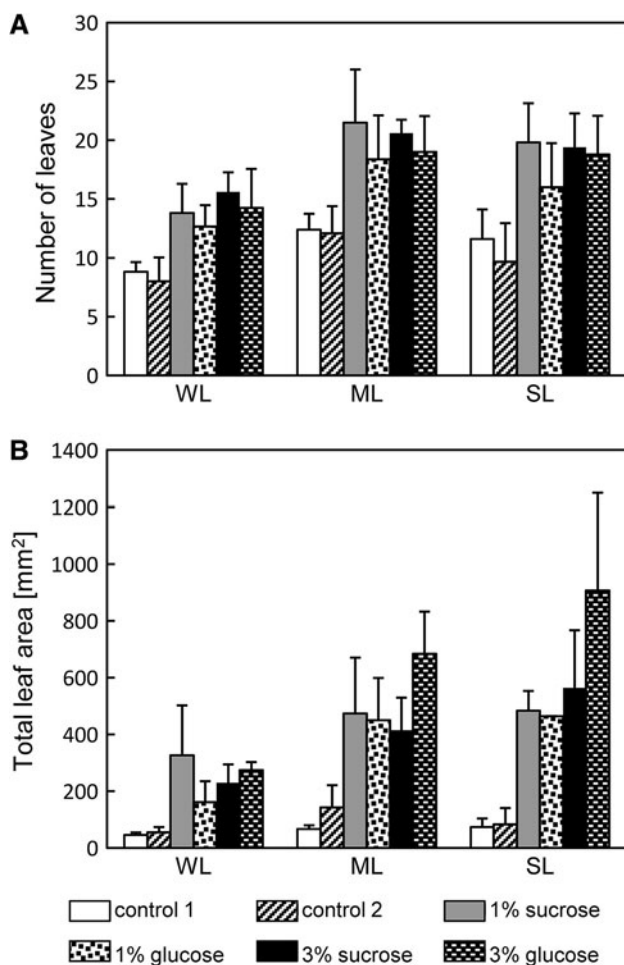


Fig. 2 Growth parameters of *Arabidopsis thaliana* cultured in vitro. **a** Number of leaves. **b** Total leaf area. Each column represents the mean value for 4–8 plants. Error bars represent SD

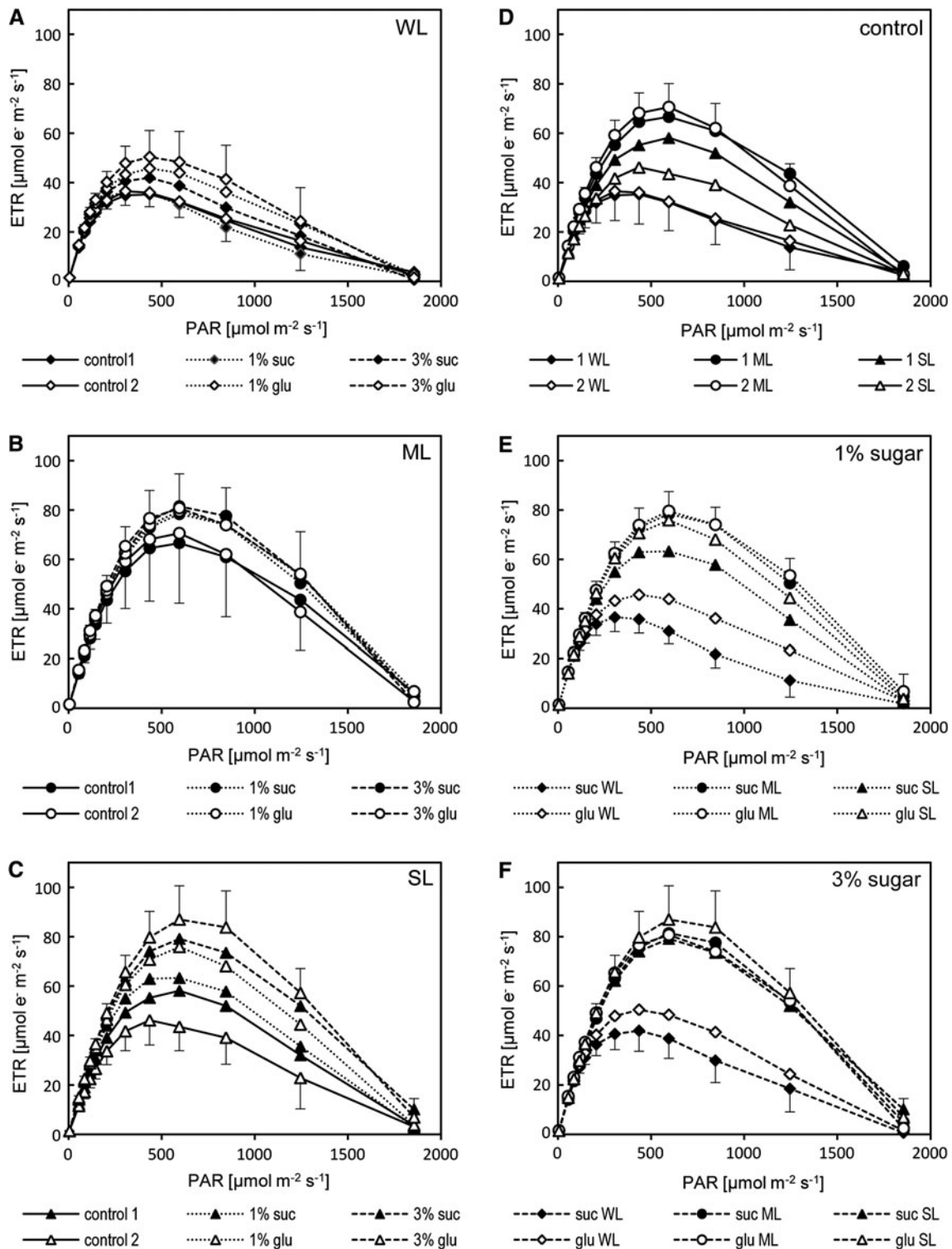


Fig. 3 Photosynthetic activity of *Arabidopsis thaliana* cultured in vitro represented as ETR light curves. **a–c** Light curves of plants cultured in different light conditions: **a** weak light, **b** medium light, **c** strong light. **d–f** Light curves of plants cultured on different media:

d control, **e** 1% sugar, **f** 3% sugar. Values are means of 10–19 measurements, carried out with different leaves from 3–6 plants. Only selected error bars representing SD have been shown to avoid obscuring the figures

As to the influence of sugar on photosynthesis, our results show a positive effect, contrary to known theories and numerous experimental works. According to Koch (1996)

exogenous sugars should cause a decrease of photosynthetic activity. This theory is based on the concept of balance between processes of sugar consumption and

production. The presence of sugar in the medium diminishes the need for sugar production and therefore should result in lower photosynthesis. This has been confirmed by several experiments performed on different plant species cultured *in vitro* (Hdider and Desjardins 1994; Van Huylenbroeck and Debergh 1996; Ryczyński and others 2007). However, there are works showing the positive influence of sugars on photosynthesis in tobacco (Tichá and others 1998; Kadleček and others 2003). Our results show that the addition of sugar to the medium enhances photosynthetic efficiency in all light conditions, but especially in SL. This means that sugar works as a photoprotectant, allowing the plant to overcome the stress and photodamage caused by SL. This effect is stronger when the sugar concentration is higher and glucose seems to be more effective. Exogenous sugar may also influence photosynthesis by enhancing plant growth and ensuring proper development in *in vitro* conditions.

Blue Light-induced Chloroplast Relocations

Chloroplast movements are one of the mechanisms that allow plants to react to changing light conditions. In most plant species chloroplast movements are induced by blue light. In response to weak light chloroplasts gather along the periclinal walls of the cell, assuming the face position. This accumulation reaction allows plants to optimize photosynthesis in conditions of light deficiency. In strong light chloroplasts gather along the anticlinal walls, in the profile position. This avoidance reaction prevents damage to the photosynthetic apparatus by excess light. The photoreceptors responsible for blue light-induced chloroplast relocations are phototropins. Phototropin 1 controls the accumulation response and phototropin 2 controls either the accumulation or the avoidance response, depending on the irradiance (Jarillo and others 2001; Briggs and Christie 2002; Kagawa and Wada 2002). Parameters that characterize chloroplast relocations can be good indicators of the cell's physiological state. Chloroplast movements require the proper functioning and cooperation of several cell components such as the cytoskeleton, the cytoplasm, signaling pathways, and energy supply mechanisms. If any of these elements is impaired in some way it will also influence chloroplast movements.

The amplitudes of both chloroplast responses to blue light were practically insensitive to sugar concentration in the medium (Fig. 4a, b). The accumulation response was only slightly stronger in plants cultured on medium containing 1% sugar than in plants cultured on medium with 3% sugar. In the case of the avoidance response, the opposite tendency was observed. Light conditions during plant culture (WL or SL) did not have a significant influence on amplitudes; however, they did have an effect on

the velocities of chloroplast movements (Fig. 4c, d). In all SL-grown plants the accumulation response was faster than in WL-grown plants. No influence of sugar content in the medium could be seen. In the case of the avoidance response, SL plants also reacted faster than WL plants grown on the same medium, but the influence of sugar concentration was visible. Plants cultured in the presence of 3% sugar had a slower avoidance response than plants cultured on 1% sugar in the same light conditions. In sucrose-grown plants velocities of the avoidance response were slightly higher than in the respective glucose-grown plants. Unfortunately, it was technically impossible to perform chloroplast movement measurements on control plants due to their small size. The average values of transmittance in dark-adapted leaves (dark transmittance, DT) used for the measurements are also shown (Fig. 4e).

The amplitudes of both responses may be influenced by the initial DT levels of leaves used in the experiment. As can be seen in Fig. 4e, the differences in DT among the different types of plants are significant and follow a certain pattern. Higher sugar concentration and SL cause a decrease in DT for both sugars. Both factors enhance plant growth resulting in thicker leaves which transmit less light. Due to these anatomical differences it was impossible to select leaves with a similar DT from all types of plants. The differences in amplitudes between plants grown on 1 and 3% sugar may be, at least partly, the result of these differences in DT. A higher DT means that the initial transmittance level is “farther” from the saturation level of the accumulation response (resulting in a higher amplitude) and “closer” to the saturation level of the avoidance response (resulting in a lower amplitude), as in the case of plants cultured on 1% sugar. Another factor influencing DT is the initial distribution of chloroplasts in dark-adapted cells, which is dependent on light conditions during plant growth (Trojan and Gabryś 1996). In WL-grown plants adapted to darkness, chloroplasts are closer to the face position, thus reducing the amplitude of the accumulation response and increasing the amplitude of the avoidance response. In SL-grown plants, chloroplasts are initially closer to the profile position. The amplitude values obtained in our experiment have probably been affected by both leaf anatomy and initial chloroplast distribution. Thus, no conclusions about the signaling role of sugar administered through the roots can be drawn from the analysis of movement amplitudes.

The velocities of both chloroplast responses are influenced by light conditions during plant growth. Chloroplasts of plants grown in SL react faster to both weak and strong blue light. This means that SL plants are better adapted to changing light conditions. The avoidance response is slowed down by the higher concentrations of both sucrose and glucose. In a previous study, it was demonstrated that

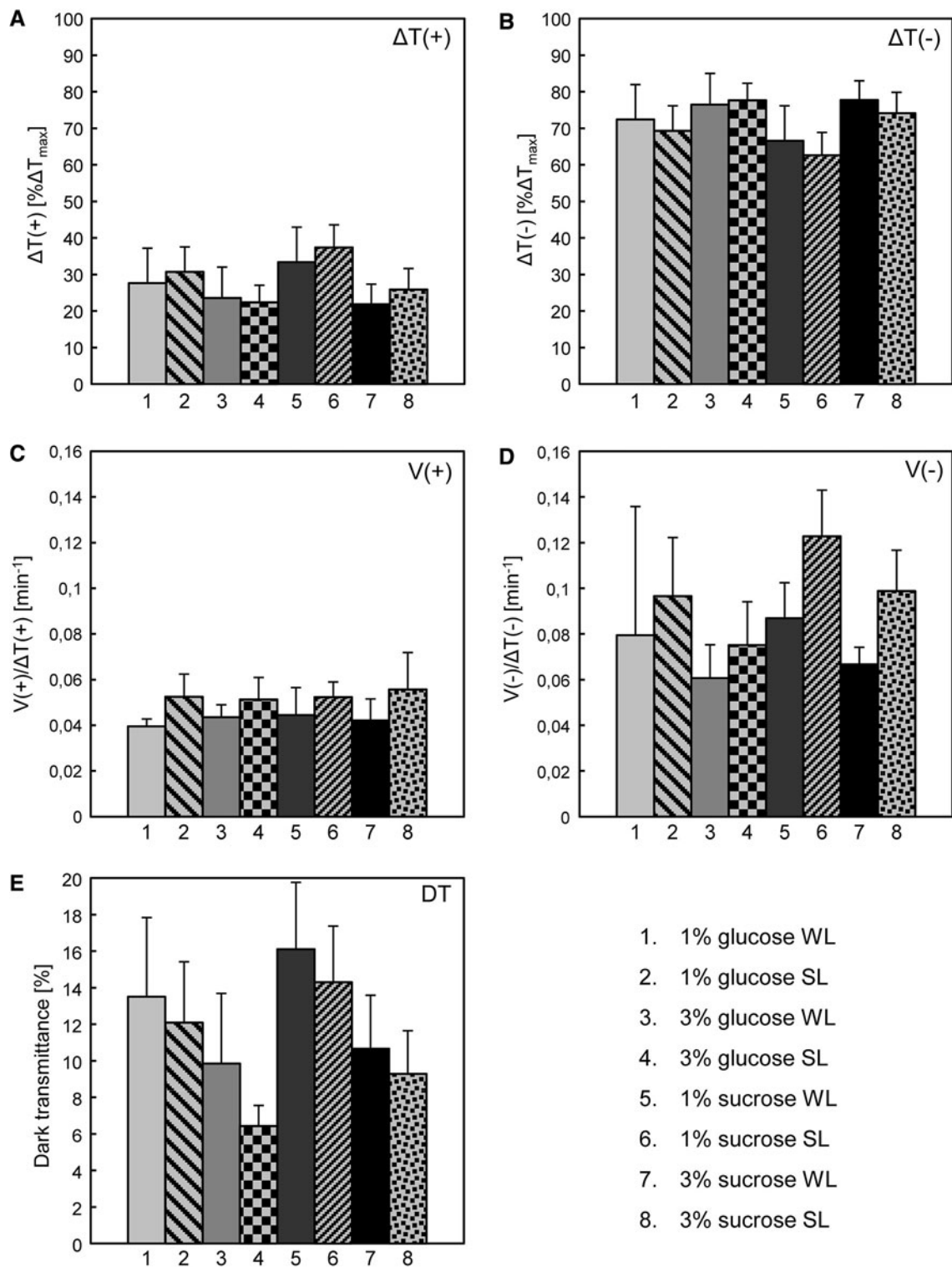


Fig. 4 Parameters of chloroplast relocations in *Arabidopsis thaliana* leaves. **a** Amplitudes of the accumulation response induced by weak blue light ($1.6 \mu mol m^{-2} s^{-1}$). **b** Amplitudes of the avoidance response induced by strong blue light ($120 \mu mol m^{-2} s^{-1}$). **c** Velocities of the accumulation response. **d** Velocities of the avoidance

response. **e** Light transmittance in dark-adapted leaves. Amplitudes are expressed as the percentage of the maximal amplitude. Velocities are normalized relative to the amplitudes. Each column represents the average of 15–23 measurements, carried out with different leaves from 5–7 plants. Error bars represent SD

sugars applied directly to the leaves inhibit chloroplast movements (Banaś and Gabryś 2007). Various sugars at different concentrations caused a strong inhibition of both chloroplast responses in *Arabidopsis*. After 3% sucrose or glucose treatment for 2 days, the amplitudes were reduced by 40–70% and velocities by 60–70%, depending on the response. Sucrose was found to have a stronger inhibitory effect than glucose. In our work, higher sugar concentration did not reduce amplitudes. A small inhibition was observed only in the velocity of the avoidance response. These results show that sugars act differently depending on the means of delivery, that is, through the root system or directly to the leaves. A strong modulatory effect of sugars on the movement apparatus is possible only in the presence of a local sugar gradient in the cell.

Xanthophyll Cycle

Figure 5 shows the changes in the de-epoxidation state (DEPS) of the xanthophyll cycle pigment pool after subsequent dark and light treatments. The DEPS parameter is calculated according to the equation $DEPS = (Zx + 0.5Ax)/(Zx + Ax + Vx)$, where Zx , Ax , and Vx are the percentages of zeaxanthin, antheraxanthin, and violaxanthin in the xanthophyll cycle pigment pool. The change in DEPS after exposure of dark-adapted plants to strong light gives a measure of the activity of the xanthophyll cycle.

All plant types exhibited a normal functioning of the xanthophyll cycle, but its activity varied depending on plant culture conditions. Plants grown in WL reached the lowest DEPS values after strong light treatment on all types of medium. ML-grown plants had the highest DEPS, and SL-grown plants cultured on 3% sugar reached the same value. In the case of 1% sugar media, plants grown on sucrose had a higher xanthophyll cycle activity than plants grown on glucose in the same light conditions. This was also the case for plants cultured on 3% sugar in WL. Plants grown on 3% sugar in ML and SL all exhibited the same xanthophyll cycle activity. In the case of controls, the xanthophyll cycle was more active in control 2 plants grown in WL and ML than in the corresponding control 1 plants.

A low xanthophyll cycle activity in WL plants is not surprising because these plants are not used to SL. Therefore, one should expect SL-grown plants to be the best adapted to these conditions and yet the xanthophyll cycle functions most efficiently in ML-grown plants. This suggests that SL during culture is a source of stress and impairs proper development. However, a positive effect of sugar present in the medium can be seen. Plants grown on sugar-containing media have a higher xanthophyll cycle activity than the respective control plants cultured in the same light conditions. At a 3% concentration sugar

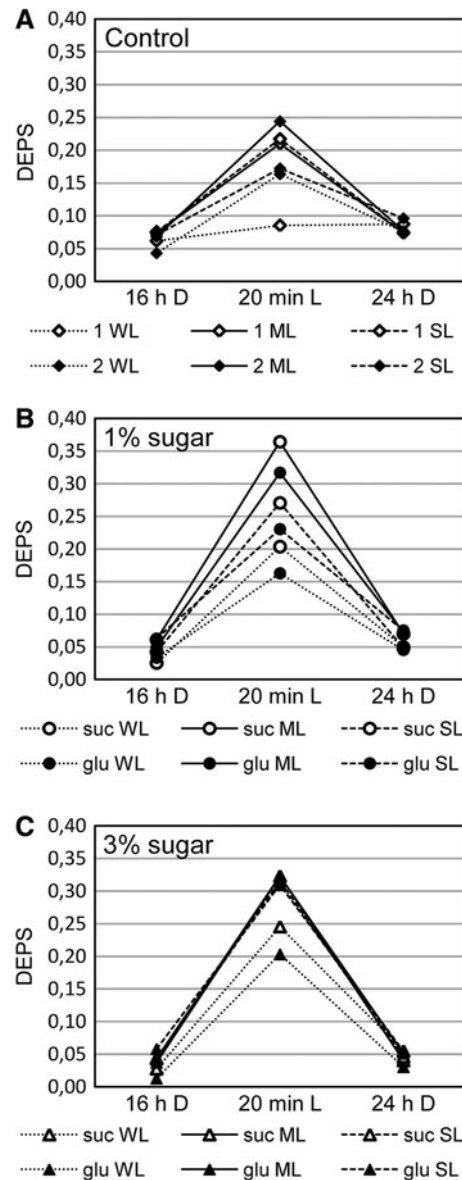


Fig. 5 Changes in the de-epoxidation state (DEPS) of the xanthophyll cycle pigment pool in *Arabidopsis thaliana* leaves after consecutive dark adaptation for 16 h, strong-light illumination for 20 min, and dark adaptation for 24 h of **a** control plants, **b** plants cultured on 1% sugar media, and **c** plants cultured on 3% sugar media. Values were calculated based on the results of HPLC analysis of 2–5 plants. Results obtained for plants of the same type were reproducible

overcomes the stress effect of SL. This is another example of the photoprotective role of sugar. In this respect sucrose seems to be more effective than glucose, especially at lower concentration. Sugar may also play a role in control plants. Higher xanthophyll cycle activity in control 2 plants can be connected to the fact that these plants were germinated on a 1% sucrose medium. This short contact with sugar may be an important signal that influences their further development.

Gene Expression

UGPI is one of the *Arabidopsis* genes encoding UDP-glucose pyrophosphorylase. Its expression is known to be specifically enhanced by sucrose (incubation of detached leaves with sugar) and light (Cierieszko and others 2001). This gene was therefore used as a marker of sucrose activity (Fig. 6a). *UGPI* expression was higher in plants grown on sucrose-containing media than in control 1 plants. The effect was concentration-dependent and additionally enhanced by SL. These results show that sucrose has the expected influence on *UGPI* expression when delivered via roots. *UGPI* expression in control 2 plants was on average lower than in plants cultured on glucose, but no specific influence of light and sugar concentration could be seen. It seems as though in the presence of glucose *UGPI* expression was dependent on different factors. *UGPI* expression in control 2 plants was relatively higher than in control 1 plants. This may be due to the contact with sucrose in the first stage of growth of control 2 plants.

CAB genes encode chlorophyll *a/b*-binding proteins. Their expression increases together with increasing photosynthetic activity. A strong upregulation by sucrose and light could be seen among sucrose-grown plants (Fig. 6b). The highest expression was achieved in 1% sucrose SL-grown plants. The higher expression in SL-grown plants is not surprising because photosynthesis is enhanced in stronger light. The upregulation of *CAB* genes by sucrose is contrary to Koch's theory of "feast" and "famine" genes, the expression of which is regulated by sugars (Krapp and others 1993; Koch 1996; Pego and others 2000). According to this model, "famine" genes connected with processes of

energetic substrate production such as photosynthesis should be downregulated in the presence of exogenous sugars. However, this result is consistent with the positive effect of sugars on photosynthesis of in vitro-cultured *Arabidopsis*. No significant differences in expression could be seen among plants grown on glucose. In control 2 plants a slight upregulation by SL could be observed. This may point to a different interaction of sucrose and glucose with light signals.

PHOT1 and *PHOT2* encode phototropins 1 and 2, respectively. The expression of *PHOT1* was enhanced by both sucrose and glucose (Fig. 6c). No influence of sugar concentration or irradiance could be observed. The slightly higher expression in control 2 plants compared to control 1 may be connected with their contact with sucrose during germination. This may mean that sugar plays a signaling role in this case. A sucrose signal received during the first stage of growth seems to influence later development.

PHOT2 expression was also upregulated by sucrose (Fig. 6d). In plants cultured on 3% sucrose *PHOT2* expression was higher in WL. In the case of glucose no influence of sugar concentration or irradiance could be observed, with expression levels being rather random. The inhibitory effect of 3% sugar on the chloroplast avoidance response does not seem to be connected with the expression levels of *PHOT1* and *PHOT2*.

VDE1 and *ABA1* encode the xanthophyll cycle enzymes violaxanthin de-epoxidase and antheraxanthin epoxidase, respectively. Both of these genes were strongly upregulated in plants cultured on 1% sugar in SL (Fig. 6e, f). However, these were not the plants with the highest xanthophyll cycle activity. In other types of plants culture conditions did not

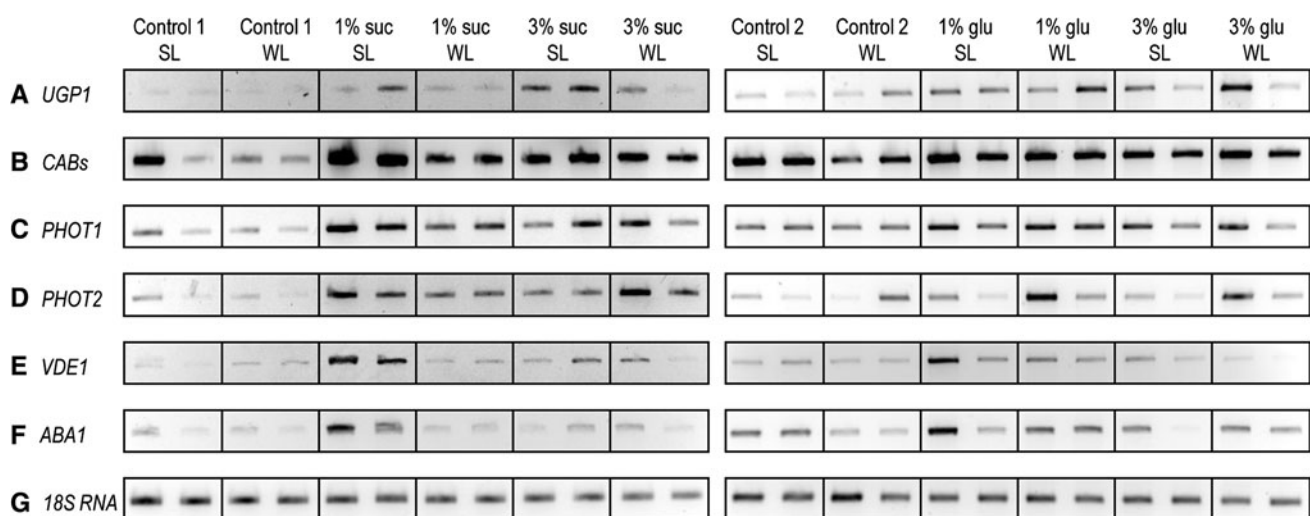


Fig. 6 RT-PCR analysis of gene expression in *Arabidopsis thaliana* cultured in vitro: **a** *UGPI*, **b** *CABs*, **c** *PHOT1*, **d** *PHOT2*, **e** *VDE1*, **f** *ABA1*, **g** *18S RNA*. Two samples from each type of plant were

analyzed. The experiment was repeated at least three times for each gene with similar results

have a visible influence on gene expression. This is not surprising because the activity of xanthophyll cycle enzymes is known to be regulated mainly at the post-transcriptional level (Jahns and others 2009). What is interesting is the upregulation by 1% sugar and SL. It seems as though this particular combination of the two factors, and not each of them separately, was a signal to enhance gene expression.

Conclusions

Arabidopsis thaliana cultured in vitro requires the presence of sugar in the medium as an additional carbon source. A 1% concentration of sucrose or glucose is sufficient for normal growth and development, but higher sugar concentrations can be beneficial in some aspects. Exogenous sugars influence physiological processes and gene expression. These signaling effects are dependent on the kind of sugar and its concentration. Sucrose present in the medium only during germination and the first two weeks of culture can affect gene expression in mature plants. Exogenous sugar enhances the photosynthetic activity of plants grown in vitro and upregulates the photosynthesis-related *CAB* genes.

Arabidopsis grows well in both ML and SL. WL significantly limits growth: the plants react less efficiently to sudden changes in irradiance (chloroplast movement, xanthophyll cycle) and easily undergo photoinhibition. Light conditions during culture influence the expression of several genes. SL can be a source of stress. Sugars play a photoprotective role: the higher concentration (3%) overcomes the negative effect of SL during culture on the functioning of photosynthesis and the xanthophyll cycle.

The chloroplast avoidance response in plants cultured on 3% sugar was slightly inhibited compared to plants grown on 1% sugar in the same light conditions. This effect is not related to changes in *PHOT1* and *PHOT2* expression.

Some combinations of sugar concentration and irradiance resulted in particularly strong and unexpected effects: exceptionally good growth of 3% glucose SL-grown plants and enhanced *VDE1* and *ABA1* expression in 1% sugar SL-grown plants. Such effects may point to interactions between sugar- and light-induced signaling pathways.

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