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BRIEF COMMUNICATION

Effect of different sugars on photosynthesis and chlorophyll fluorescence in photoautotrophic tomato suspension cell cultures

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Abstract

The effects of metabolisable sugars sucrose and glucose along with non-metabolisable isomers of sucrose palatinose and turanose were tested. Rate of oxygen evolution (*P*), electron transport rate (ETR), and photochemical quenching (q_P) showed substantial decrease after 24 and 48 h by glucose and sucrose treatments, whereas there was no effect on all these parameters by the treatment with palatinose and turanose. Also the F_v/F_m ratio remained constant through the time of studies revealing that the maximal photochemical capacity of the cells was unchanged. Non-photochemical quenching (q_N) showed a decrease compared to the control values by all the treatments. Hence *P* and Chl fluorescence parameter were affected only by those sugars which are used in the metabolic pathways and not by sugar analogues.

Additional key words: electron transport rate; glucose; *Lycopersicon peruvianum*; oxygen evolution; palatinose; saccharose; turanose.

Photoautorophic suspension cell cultures are an excellent material to carry out various plant science studies. An increasing number of photoautotrophic cultures is established (Mühlbach 1998). In this report we used the established photoautotrophic suspension cell culture of tomato (*Lycopersicon peruvianum* L., Beimen *et al.* 1992) to study the effect of different sugars, metabolisable and non-metabolisable, on rate of oxygen evolution (*P*) and different chlorophyll (Chl) fluorescence parameters. Sugars not only provide growth and development of the plants but also regulate various genes (Koch 1996). Sugars affect transcription levels of various sink and source specific enzymes (Ehneß and Roitsch 1997, Ehneß *et al.* 1997, Godt and Roitsch 1997). Sugars also co-ordinately regulate the transcription levels with stress-related stimuli and hormones (Ehneß and Roitsch 1997, Roitsch 1999, Sheen *et al.* 1999). Using sucrose analogues palatinose and turanose along with other sugars, Loreti *et al.* (2000) showed differential effects on the α-amylase expression from barley embryos. Using palatinose, Fernie *et al.* (2001) found effects similar to that of sucrose on the degradation of sucrose and starch in potato tubers. Herold *et al.* (1980) and Foyer (1988) showed feedback inhibition of photosynthesis by feeding excessive sugars to leaves. In the photoautotrophic suspension culture cells of *Chenopodium rubrum*, feeding glucose resulted in the decrease of Chl content and accumulation of saccharides (Schäfer *et al.* 1992) and a slight decrease in maximum photosynthetic efficiency, as determined by Chl fluorescence and rate of photosynthesis.

Photoautotrophic cell suspension culture cells of *Lycopersicon peruvianum* L. were established by Beimen *et al.* (1992). They were sub-cultured every two weeks in MS-medium and are incubated by shaking under continuous irradiation with an atmosphere containing 2 % CO_2. *P* of the cell cultures was measured using a liquid phase oxygen electrode (*Frank Bros.*, Cambridge, UK) at saturating irradiance provided by halogen lamp projector. The cells were first allowed to respire for 1 min and then irradiated for the measurement of *P*. Equal volume of cells was used each time and immediately after the measurments, the cells were taken out carefully and fresh mass was determined. Modulated Chl fluorescence of the tomato cell suspension culture were measured using

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a *PAM-2000* chlorophyll fluorometer (*H. Walz*, Effeltrich, Germany). Maximum photosystem 2 (PS2) quantum yield of a dark adapted sample, F_v/F_m, and q_P and q_N (for nomenclature see van Kooten and Snel 1990) were measured on cells dark-adapted for 15 min as described by Schreiber *et al.* (1986) using a pre-programmed protocol (*Standard Run 3*). The suspension cells were taken in a special cuvette and kept stirring throughout the measurement. Initial Chl fluorescence was measured using a weak modulated red radiation. Maximum fluorescence was measured after a 0.8 s pulse of strong "white light" [>4 000 μmol(photon) m^{-2} s^{-1}(PAR)]. After a 2 s lag, a 5 min quenching analysis was initiated using a continuous actinic radiation [125 μmol(photon) m^{-2} s^{-1}(PAR) emitted at 665 nm] and saturating pulses of 0.8 s every 20 s. The steady-state value obtained at the end of Run 3 were reported as the values of F_v/F_m and quenching parameters. The ETR_{max} reported were measured using Light Curve programme from a newly developed *PAM-WIN* software, based on the parameters reported by Genty *et al.* (1989) and compatible with *PAM-2000* fluorometer.

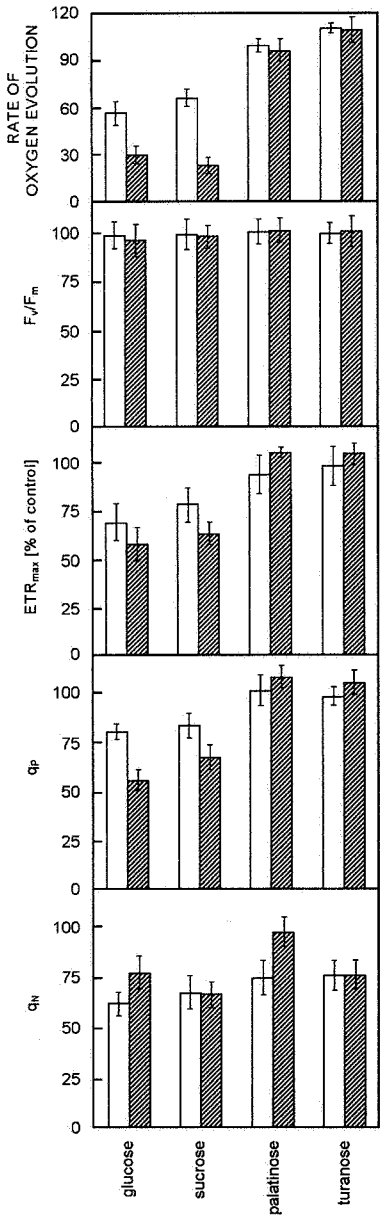

Fig. 1. The rate of oxygen evolution and different chlorophyll fluorescence parameters after feeding with different sugars at 24 h (*white bars*) and 48 h (*grey bars*). Means of six different experiments in percent of control.

The effect of different sugars (concentration of 50 mM) on *P* and parameters of Chl fluorescence was tested after 24 and 48 h of the treatments. The same population of cells without any treatment was taken as control. Palatinose and turanose are structural isomers of sucrose and are composed of glucose and fructose in α-glycosidic linkage. These are not synthesised by higher plants and cannot be cleaved either by invertase or sucrose synthase or any other known plant enzyme. Use of these sugars may give an indication whether the metabolisable sugars and its non-metabolisable forms have the same or different effects.

With glucose treatment the *P* decreased after 24 h and substantially after 48 h (Fig. 1). Earlier time point studies revealed slight but not significant changes in the rates (values not shown). Sucrose treatment also showed a repression in *P* but it was more pronounced at the end of 48 h as compared to glucose (Fig. 1). The non-metabolisable sucrose analogues, palatinose and turanose, had no effect on the rate of oxygen evolution at the time points studied.

Among the Chl fluorescence parameters the optimal fluorescence yield F_v/F_m remained unchanged at both time points of studies by all sugars under investigation (Fig. 1). The maximum ETR (as determined by Chl fluorescence) and q_P showed a decrease of 40 % by glucose treatment and 30 % by sucrose treatment. There was no effect on these two parameters by turanose and palatinose treatments. The q_N showed a decreased value as compared to control cells at all the time points of studies and also by all the sugars tested (Fig. 1).

The maximum ETR rate was deduced from the irradiance curve programme of newly developed *PAM-WIN* software. The cells were first dark-adapted for 15 min and then the irradiance curve programme was initiated. It measures the rate of electron transport at different photosynthetically active radiation (PAR) and from the curve obtained it calculates the maximum ETR. A typical irradiance curves after 48 h treatments with glucose and turanose were compared with that of control (Fig. 2). A significant decrease in the irradiance curve obtained by glucose treatment was observed while there was no effect by turanose treatment. The results with palatinose treatment were similar to that of turanose (values not shown).

The feedback inhibition of photosynthesis by feeding sugars is known (Herold *et al.* 1980, Foyer 1988, Morcuende *et al.* 1997). Sugars repress the transcript level of

many genes involved in the photosynthetic pathway (Koch 1996, Roitsch 1999). There are also indications of cross talks between sugar signals, stress stimuli, and hormone effects (Roitsch 1999, Sheen *et al.* 1999). We compared the effect of metabolisable with non-metabolisable sugars on the rate of photosynthetic oxygen evolution and on Chl fluorescence parameters. Turanose and palatinose are not recognised or transported by sucrose transporter (M'Batchi *et al.* 1984, M'Batchi and Delrot 1988, Li *et al.* 1994). Fernie *et al.* (2001) showed poor absorption of palatinose by slices of potato tubers.

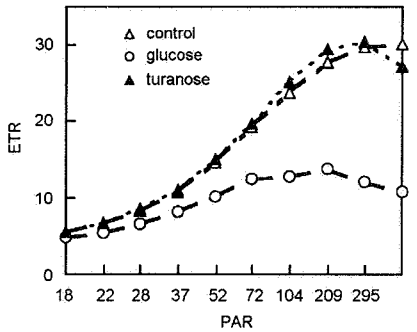

Fig. 2. A typical irradiance (PAR) curve of electron transport rate (ETR) after 48 h of feeding glucose (○) and turanose (●) compared with control (Δ).

The inhibition of rate of photosynthesis was observed only by feeding glucose and sucrose. The substantial decrease in rates was observed 24 h after adding these sugars. Glucose feeding in *C. rubrum* culture a *ca.* 70 % decrease in the rate of oxygen evolution (Schäfer *et al.* 1992) after 3-4 d. The fact that rates were not decreased by the non-metabolisable sucrose isomer reveals that only those sugars that are taken up by the cells and used up in further metabolic pathways cause reduction in rate of photosynthesis. The amount of turanose and palatinose remained constant in the culture supernatant even after 48 h while at the same time period al-most 80-85 % of glucose and sucrose were consumed (values not shown).

F_v/F_m after dark adaptation remained unchanged by all the treatments and hence the photochemical capacity of the cells remained unchanged. Again, only the effective quantum yield, which in this case was demonstrated by the rate of ETR_{max}, was reduced by glucose and sucrose treatments. Under stresses such as low temperature (Brüggemann *et al.* 1992), heat (Bilger *et al.* 1987, Law and Crafts-Brandner 1999), drought and salinity (Cornic and Briantais 1991), a decrease only in the effective quantum yield and not in the maximal quantum yield is usually observed (Schreiber and Bilger 1993). Our results obtained with sugars were in agreement with the other stress studies. q_P measured by the saturating pulse method (Schreiber and Bilger 1993, Schreiber *et al.* 1994, 1995) showed decrease by glucose and sucrose treatments only. This again supports the results of *P* studies. In tomato suspension cell cultures we observed substantial decrease in q_P only after 24 h, which is in contrast to what was observed by stress related stimuli such as mechanical wounding, current application, and heat stress (Krause and Weis 1991, Fishan *et al.* 1995, Herde *et al.* 1999). The decrease in photochemical quenching is correlated with the decrease in the quantum efficiency of light-driven electron transport through PS2. Therefore during the steady-state photosynthesis a stoichiometric balance between the reactions that consume and those that produce NADPH was maintained by decreasing the photochemical quenching (Weis and Berry 1987, Woodrow and Berry 1988, Schreiber and Bilger 1993). This was shown in the irradiance curve experiment where the decrease in the rate of carbon fixation by glucose was reflected by decrease in electron transport in PS2, whereas neither the irradiance curve nor the carbon fixation rate was affected by turanose treatment. Surprisingly we did not observe any increase in q_N, but found a decrease by all the sugars. A wide variety of stresses increases q_N (Pfündel and Bilger 1994, Herde *et al.* 1999). As other studies were carried on in intact plant leaves and our system was a photoautotrophic suspension cell culture, this needs further investigation. The relaxation kinetic studied for the quenching analysis (values not shown) showed a fast recovery with all the sugars indicating that the quenching was energy dependent.

The results indicate that the co-repression of photosynthetic genes observed in the presence of sugars is reflected by changes in the rate of photosynthesis and Chl fluorescence parameters. The effect of palatinose and turanose is considered as an evidence for extra-cellular sugar sensing by Loreti *et al.* (2000) and Fernie *et al.* (2001). The differential effect in comparison to metabolisable sugars raises the question of the suitability of palatinose and turanose as appropriate tool to study sugar sensing. Our study indicates that closely related saccharides may have different effect on signalling pathways. Though the current studies are confined to physiological values, more studies to analyse the effect at molecular and biochemical levels are under way.

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