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Light regulation of diurnal variation of sucrose-phosphate synthase in potato

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Abstract

Light regulation of diurnal variation of sucrose-phosphate synthase (SPS) activity was studied in potato leaves during pre-tuber initiation stage. SPS activity was greatest at 14:00 and coincident with highest irradiance [2 000 μmol(PAR) m^{-2} s^{-1}], and lowest at 18:00 during lowest irradiance (200 μmol m^{-2} s^{-1}). In contrast, fructose-1,6-bisphosphatase (FBPase) activity was greatest at 12:00 (irradiance 1 700 μmol m^{-2} s^{-1}). Inhibition of SPS activity by phosphorylation was least at 14:00 and highest at 18:00. Considerable inhibition was also observed at 10:00. SPS showed hyperbolic saturation kinetics in response to varying UDP-glucose concentrations. Diurnal variation of SPS activity was due to a change of affinity for its substrate (K_m), but not due to change of V_{max}. Phosphate (P_i) decreased the activation state of SPS. Inhibition by P_i was most severe under limiting UDP-glucose concentration. Sensitivity towards P_i inhibition at limiting substrate concentration was highest at 18:00 and lowest at 14:00. Change of tissue phosphate concentration followed the opposite pattern to that of diurnal variation of SPS activity and irradiance, being highest at 18:00 and lowest at 14:00. A dual mode of regulation is considered to exist for diurnal variation of potato leaf SPS: covalent modification and effector (P_i) regulation. Thus the change of tissue phosphate concentration may play a pivotal role in regulating potato leaf SPS.

Additional key words: activation state, covalent modification, irradiance, *Solanum tuberosum* L., substrate affinity.

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Abbreviations: EDTA - ethylenediaminetetraacetic acid; FBPase - fructose-1,6-bisphosphatase; f.m. - fresh mass; PMSF - phenylmethylsulfonyl fluoride; PVP - polyvinyl pyrrolidone; SPS - sucrose-phosphate synthase; TCA - trichloroacetic acid.

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Introduction

In plants, the formation of sucrose is regulated at two steps: reaction catalyzed by fructose-1,6-bisphosphatase (FBPase) and that of sucrose-phosphate synthase (SPS). The reaction catalyzed by SPS plays a pivotal role in photosynthetic sucrose formation. Transgenic tomato plants expressing high activity of maize SPS reached saturation of net photosynthetic rate at higher CO_2 concentration (Worrel *et al.* 1991, Galtier *et al.* 1993) and had lower concentration of starch and higher concentration of sucrose in leaves (Worrel *et al.* 1991). SPS activity is found to vary diurnally in many species in response to light-dark transition (Pollock and Housley 1985, Sicher and Kremer 1985, Stitt *et al.* 1988, Huber *et al.* 1989). Light is found to activate SPS and light modulation of SPS activity involves covalent modification by phosphorylation-dephosphorylation and a change of sensitivity towards allosteric effectors, inorganic phosphate (P_i) and glucose-6-phosphate (Stitt *et al.* 1987, 1988, Huber and Huber 1996).

In potato photoassimilates are mostly translocated in the form of sucrose and stored in tubers. So, elucidation of the mechanisms by which SPS is regulated *in vivo* is interesting namely in potato in which very little work has been carried out. The purpose of the present study was to investigate the mechanism of diurnal variation of SPS activity.

Materials and methods

Plants: Potato (*Solanum tuberosum* L.) cv. Kufri Chandramukhi plants were grown in pots in glasshouse under natural irradiance in Shimla (14-h photoperiod, day/night temperature 25 - 28/15 - 18 °C, relative humidity of air 60 - 70 %). The irradiance increased from 900 μmol (PAR) m^{-2} s^{-1} at 08:30 to the maximum (2 000 μmol m^{-2} s^{-1}) at 14:00 and decreased to 200 μmol m^{-2} s^{-1} at 18:00. Tubers of this cultivar were obtained from Genetics Division, CPRI, Shimla. The fully expanded leaves of 20- to 40-d-old plants (pre-tuber initiation stage) were used for analyses.

Enzyme extraction: Leaves were harvested at different times during the day and washed with distilled water. Leaves were homogenized in precooled mortar and pestle in four volumes (m/v) of 50 mM Hepes-NaOH buffer (pH 7.5), which comprised 1 mM EDTA, 10 mM β-mercaptoethanol (*Sigma*, St. Louis, USA), 10 mM NaF, 1 mM PMSF (buffer A) and 0.3 g(PVP) g^{-1}(leaf tissue), and the homogenate was filtered through four layers of muslin cloth in a cold room at 4 °C. The enzyme filtrate was centrifuged at 18 000 *g* for 10 min at 4 °C. The soluble fraction was passed through *Sephadex G-25* column (except in case of tissue phosphate determination), preequilibrated with buffer A. The desalted extract was used for all enzymatic assays.

SPS assay: The procedure of Cheikh and Brenner (1992) was followed. The reaction mixture consisted of 50 mM Hepes-NaOH (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA,

10 mM fructose-6-phosphate (*Sigma*), 10 mM UDP-glucose (unless otherwise mentioned) and 0.3 cm^3 of enzyme extract in a total volume of 0.6 cm^3. Reaction was carried out at 30 °C for 30 min and terminated by addition of 0.1 cm^3 of 1M NaOH and then kept in boiling water bath for 10 min. After keeping the reaction tubes in ice bath for few minutes 2 cm^3 of ice cold 0.1 % (m/v) anthrone in 76 % (v/v) H_2SO_4 was added and mixed thoroughly by vortexing. Colour was developed by keeping the test tube in water bath for 15 min at 40 °C. Absorbance was measured at 620 nm.

Covalent phosphorylation status of SPS at different irradiances was studied by preincubating the desalted enzyme extract in 2 mM ATP (enzyme extract:ATP - 5:1, v/v) for 15 min at 20 °C. The preincubation medium contained 50 mM Hepes-NaOH (pH 7.5), 1 mM PMSF (*Sigma*) and was used with and without 10 mM $MgCl_2$. No ATP was added in control. After preincubation an aliquot (0.03 cm^3) of the solution was diluted 10 folds with 50 mM Hepes-NaOH (pH 7.5) and SPS activity was measured as described above.

The change of kinetic response of SPS during three different times of the day was studied by varying UDP-glucose concentration from 5 to 40 mM. Phosphate inhibition of SPS activity was studied at two UDP-glucose concentrations, 10 mM and 40 mM. NaH_2PO_4 (10 mM) was included in the assay buffer and SPS activity was measured.

FBPase assay: Cytoplasmic FBPase was assayed spectrophotometrically by measuring fructose-1,6-bisphosphate dependent P_i release (Cheikh and Brenner 1992). The reaction mixture contained 50 mM Hepes-NaOH (pH 7.5), 10 mM NaF, 10 mM $MgCl_2$, 1 mM PMSF, 0.25 mM fructose-1,6-bisphosphate and 0.2 cm^3 of enzyme extract in a final volume of 0.5 cm^3. Reaction mixture was incubated at 30 °C for 30 min. Activity of non-specific phosphatase was estimated by omission of $MgCl_2$ from the reaction mixture. The reaction was terminated by addition of 1 cm^3 of ice cold 30 % (m/v) TCA and then kept in an ice bath for 5 min. Aliquot was then centrifuged at 10 000 *g* for 5 min. P_i was determined following the procedure of Rathbun and Betlach (1967). One cm^3 of a mixture of acetate buffer [1:1 (v/v) mixture of 3 M acetic acid and 3 M sodium acetate] and formaldehyde (1.0:0.1, v/v) was quickly mixed with the aliquot. The molybdenum blue colour was developed by quick addition of 0.1 cm^3 of 2 % (m/v) ammonium molybdate and 0.2 cm^3 of 6.75 mM $SnCl_2$ to each aliquot which was quickly mixed and allowed to stand at room temperature for 15 min. Absorbance was measured at 735 nm.

Tissue phosphate concentration: Total tissue phosphate was estimated from the crude leaf extract without passing through *Sephadex* column (*Pharmacia*, Uppsala, Sweden). One cm^3 of 30 % (m/v) ice cold TCA was added to 0.2 cm^3 of leaf extract and kept in ice bath for 5 min. Remaining procedure was the same as described for P_i determination above.

Results

SPS activity in potato leaves increased steadily during the day time and attained a peak at 14:00 during the period of highest irradiance [2 000 μmol (PAR) m^{-2} s^{-1}] followed by sharp decline to its lowest activity at 18:00 (irradiance 200 μmol m^{-2} s^{-1}) (Fig. 1). The activity at 14:00 was about 200 % higher than the activity found at 10:00 in the morning (irradiance 1 500 μmol m^{-2} s^{-1}).

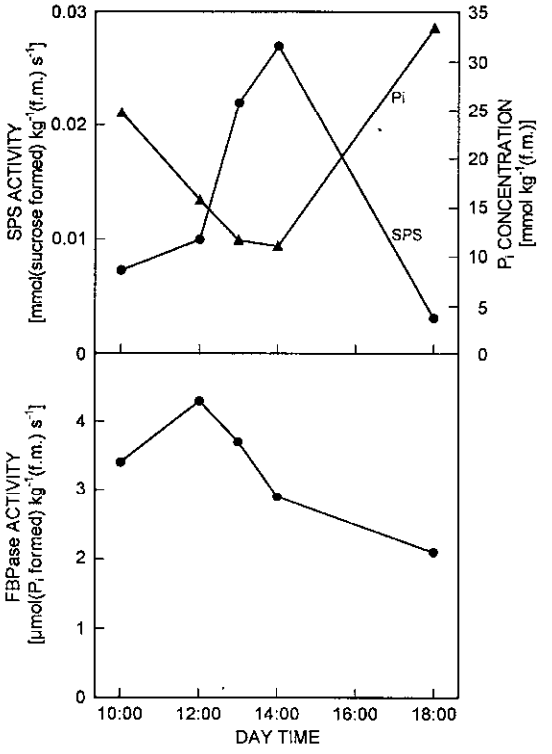

Fig. 1. Diurnal variation of potato leaf sucrose-phosphate synthase (SPS) and fructose-1,6-bis-phosphatase (FBPase) activity and concentration of inorganic phosphate (P_i) (irradiance 1 500, 1 700, 1 900, 2 000, 200 μmol m^{-2} s^{-1} at 10:00, 12:00, 13:00, 14:00 and 18:00, respectively). Means of three independent determinations. SE was lower than 10 % for all the means.

Diurnal variation of tissue phosphate concentration followed exactly the opposite pattern as that of diurnal variation of SPS activity. Tissue phosphate concentration decreased with time of a day and reached the lowest level at 14:00 during highest irradiance and thereafter increased again to the highest concentration at 18:00 (Fig. 1). FBPase activity showed much less diurnal variation than that of SPS activity (Fig. 1). FBPase activity was found to be the highest at noon (irradiance 1 700 μmol m^{-2} s^{-1}) and thereafter the activity declined steadily to its lowest level at 18:00.

The degree of inhibition of SPS activity by covalent modification (phosphorylation) was also found to vary. SPS was least sensitive to phosphorylation inhibition at 14:00. The magnitude of inhibition was 22 % in comparison to control when assayed in presence of Mg^{2+} (Table 1). The highest inhibition (88 %) was found at 18:00.

Table 1. Inactivation of potato leaf SPS (means of three independent observations ± SE) by 2 mM ATP in presence or absence of 10 mM $MgCl_2$ and change of substrate affinity of potato leaf SPS at 10:00, 14:00 and 18:00. The K_m (UDP-glucose) [mM] and V_{max} [mmol(sucrose) kg^{-1}(f.m.) s^{-1}] were calculated from the data in Fig. 2.

Day time	Irradiance [μmol m^{-2} s^{-1}]	Inactivation [%] -$MgCl_2$	+$MgCl_2$	SPS K_m	V_{max}
10:00	1 500	20 ± 2.18	74 ± 1.8	9.6	0.03
14:00	2 000	5 ± 1.46	22 ± 1.5	7.0	0.03
18:00	200	54 ± 1.67	88 ± 1.45	19.8	0.03

SPS, assayed at three different times of the day, showed hyperbolic saturation kinetics when the UDP-glucose concentration was varied from 5 to 40 mM. The enzyme from leaves harvested at the highest irradiance had higher activity than that from leaves harvested at lower irradiances (Fig. 2). The light modulation of SPS activity was due to change in apparent K_m values for UDP-glucose. V_{max} values were essentially the same in all the cases (Table 1). Enzyme from leaves harvested at 14:00 had lowest K_m value for UDP-glucose showing highest affinity for its substrate. The change in apparent K_m for UDP-glucose increased from 7.0 mM observed at 14:00 during peak activity to 19.8 mM at 18:00 during the lowest activity (Table 1).

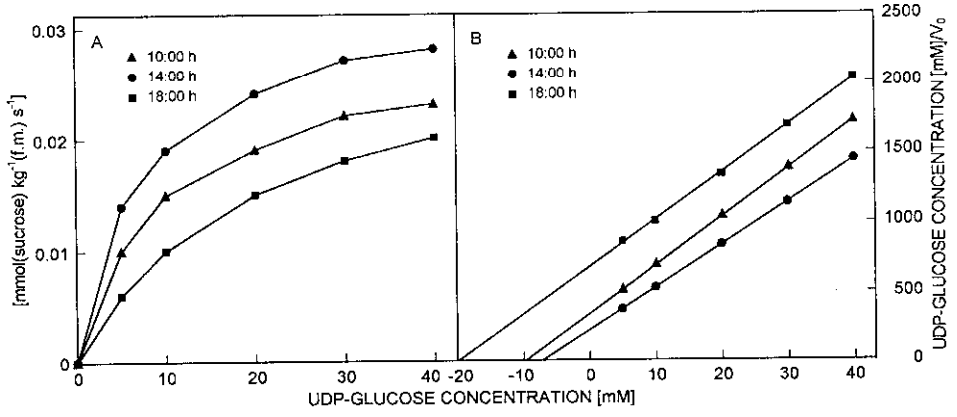

Fig. 2*A*. Response of potato leaf SPS activity to varying UDP-glucose concentrations at three different irradiances (1 500, 2 000 and 200 μmol m^{-2} s^{-1}). Means of two independent determinations. Fig. 2*B*. Replot of the results as Hanes Plot with lines fitted by linear regression to calculate the values for K_m and V_{max}.

P_i acts as an inhibitor of SPS. The degree of P_i inhibition was expressed as a change of activation state of SPS. Activation state of SPS was calculated as the ratio of activity observed in presence of P_i over activity obtained with 40 mM UDP-glucose in absence of P_i at 14:00. P_i inhibition of SPS activity was found to be maximum at limiting substrate concentration. At 10 mM UDP-glucose, SPS activity was almost completely inhibited at 18:00 whereas enzymes from leaves harvested at 10:00 and 14:00 were inhibited by 78 % and 63 %, respectively. However, the degree of inhibition was much less under saturating (40 mM) UDP-glucose concentration. At 40 mM UDP-glucose degree of inhibition was 9 % in the afternoon during peak activity and 20 - 22 % during morning and evening (Fig. 3).

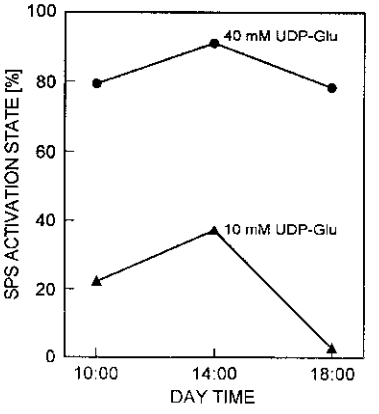

Fig. 3. Change of activation state of potato leaf SPS by P_i at two different UDP-glucose concentrations during three different times of the day. Means of three independent determinations. SE was lower than 10 % for all the means.

Discussion

The activity of SPS was found to change rapidly under a light-dark regime (Huber *et al.* 1989, 1992). It has been proposed that light activation of SPS can only be observed *in vitro* when the enzyme is assayed under limiting substrate concentration (Huber *et al.* 1992). So, the diurnal variation of SPS activity in potato was studied with 10 mM UDP-glucose which is found to be limiting for potato SPS (discussed later). Under limiting substrate concentration SPS activity showed marked diurnal fluctuation with peak activity attained at the highest irradiance. So, potato leaf SPS requires a high irradiance for saturation. This is in agreement with results obtained from other plant species that maximum SPS activity is coincident with highest irradiance (Pollock and Housley 1985, Sicher and Kremer 1985, Kalt-Torres *et al.* 1987b).

Compared to the distinct diurnal variation of SPS activity, potato leaf FBPase exhibited much less diurnal fluctuation. FBPase did not exhibit a distinct diurnal rhythm in soybean also (Rufty *et al.* 1983). The increased generation of fructose-6-phosphate by FBPase is accompanied by increased glucose-6-phosphate, which increases affinity of SPS for its substrates, UDP-glucose and fructose-6-phosphate,

and decreased P_i, a competitive inhibitor of SPS (Huber and Huber 1996). Fructose-6-phosphate has to be converted to UDP-glucose to be used by SPS. So, the observed time lag between peak activities of FBPase and SPS seems justified.

In many plant species SPS is found to be covalently modified by protein phosphorylation-dephosphorylation in response to a light-dark transition (Huber and Huber 1996). In dark SPS is phosphorylated/inactivated by a specific protein kinase at ser-158 which is present at the allosteric site of the enzyme (McMichael *et al.* 1993). Upon illumination the phospho-SPS is dephosphorylated/activated by a type 2A protein phosphatase (Huber and Huber 1992a). Phosphorylation inhibition pattern of potato leaf SPS correlates well with the diurnal variation of SPS activity, being maximum during morning and evening. So, SPS kinase remains very active both in the morning and evening. The magnitude of inhibition (covalent modification by phosphorylation) was found to be not strictly dependent upon divalent cation, Mg^{2+}. Two forms of SPS protein kinase with apparent molecular masses of 45 and 150 kDa had been purified from spinach leaves by McMichael *et al.* (1995). The smaller kinase is found to be strictly divalent cation dependent, while larger kinase is independent on the presence of divalent cation (McMichael *et al.* 1995). It could be possible that both of these forms of SPS protein kinase are also present in potato which view is supported by the high inhibition in presence of Mg^{2+}.

The hyperbolic substrate saturation kinetics of SPS for UDP-glucose is consistent with the results obtained from other plant species (Stitt *et al.* 1988, Lunn and Hatch 1997). SPS in potato leaf is found to attain apparent V_{max} at the high UDP-glucose concentration of 40 mM or more. This is in agreement with results obtained from maize leaf SPS by Lunn and Hatch (1997). The apparent K_m values of SPS have been reported to change from 1.0 to 12.9 mM in the light and from 1.7 to 18 mM in the dark (Huber *et al.* 1987, Kalt-Torres *et al.* 1987a). So, the change of K_m values of potato leaf SPS are in the same range as those previously reported. It has been observed that phosphorylation of SPS has no effect on maximum catalytic activity but involves changes in substrate affinity (Stitt *et al.* 1988). The parallel change in the K_m, but not in the V_{max}, with the change in the degree of inhibition (phosphorylation) observed in our work documents that statement.

Phosphorylation of SPS increases its affinity for the negative allosteric effector, P_i, (Huber and Huber 1992b, 1996). Consequently, the highest degree of P_i inhibition (lowest activation state) was observed in potato leaf SPS when the enzyme was highly phosphorylated (the highest inhibition by ATP) at 18:00 (the lowest irradiance). The rate of inhibition of SPS activity by ATP was coincident with the amount of tissue P_i. It is likely that in leaves increase in P_i would correspond to decrease in concentration of phosphate esters such as glucose-6-phosphate to maintain a constant phosphate pool in the cytosol (Heber and Heldt 1981). Glucose-6-phosphate was found to be an inhibitor of SPS-kinase and P_i was an inhibitor of SPS-phosphatase (Weiner *et al.* 1992). So, P_i plays a dual role in fine tuning of SPS. Apart from being a negative effector of SPS, it also regulates the covalent modification of SPS. From the opposite pattern of change of diurnal variation of SPS activity and change of tissue P_i level, it is justified to propose that change in tissue P_i concentration plays a pivotal role in regulating the activation of SPS *in situ*.

References

Cheikh, N., Brenner, M.L.: Regulation of key enzymes of sucrose biosynthesis in soybean leaves. Effect of dark and light conditions and role of gibberellins and abscisic acid. - Plant Physiol. **100**: 1230-1237, 1992.

Galtier, N., Foyer, C.H., Huber, J., Voelker, T.A., Huber, S.C.: Effect of elevated sucrose-phosphate synthase activity on photosynthesis, assimilate partitioning, and growth in tomato (*Lycopersicon esculentum* var UC82B). - Plant Physiol. **101**: 535-543, 1993.

Heber, U., Heldt, H.W.: The chloroplast envelope: structure, function and role in plant metabolism. - Annu. Rev. Plant Physiol. **32**: 139-168, 1981.

Huber, J.L.A., Huber, S.C.: Site-specific serine phosphorylation of spinach leaf sucrose-phosphate synthase. - Biochem. J. **283**: 877-882, 1992b.

Huber, S.C., Huber, J.L.: Role of sucrose-phosphate synthase in sucrose metabolism in leaves. - Plant Physiol. **99**: 1275-1278, 1992a.

Huber, S.C., Huber, J.L.: Role and regulation of sucrose-phosphate synthase in higher plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **47**: 431-444, 1996.

Huber, S.C., Huber, J.L., Campbell, W.H., Redinbaugh, M.G.: Comparative studies of the light modulation of nitrate reductase and sucrose-phosphate synthase activities in spinach leaves. - Plant Physiol. **100**: 706-772, 1992.

Huber, S.C., Nielsen, T.H., Huber, J.L., Pharr, D.M.: Variation among species in light activation of sucrose-phosphate synthase. - Plant Cell Physiol. **30**: 277-285, 1989.

Kalt-Torres, W., Kerr, P.S., Huber, S.C.: Isolation and characterization of multiple forms of maize leaf sucrose-phosphate synthase. - Physiol. Plant. **70**: 653-658, 1987a.

Kalt-Torres, W., Kerr, P.S., Usuda, H., Huber, S.C.: Diurnal changes in maize leaf photosynthesis. 1. Carbon exchange rate, assimilate export rate, and enzyme activities. - Plant Physiol. **83**: 283-288, 1987b.

Lunn, J.E., Hatch, M.D.: The role of sucrose-phosphate synthase in the control of photosynthate partitioning in *Zea mays* leaves. - Aust. J. Plant Physiol. **24**: 1-8, 1997.

McMichael, R.W., Jr., Klein, R.R., Salvucci, M.E., Huber, S.C.: Identification of the major regulatory phosphorylation site in sucrose-phosphate synthase. - Arch. Biochem. Biophys. **307**: 248-252, 1993.

McMichael, R.W., Jr., Kochansky, J., Klein, R.R., Huber, S.C.: Characterization of the substrate specificity of sucrose-phosphate synthase protein kinase. - Arch. Biochem. Biophys. **321**: 71-75, 1995.

Pollock, C.J., Housley, T.L.: Light induced increase in sucrose-phosphate synthase activity in leaves of *Lolium temulentum*. - Ann. Bot. **55**: 593-596, 1985.

Rathbun, W.B., Betlach, M.V.: Estimation of enzymatically produced orthophosphate in the presence of cysteine and adenosine triphosphate. - Anal. Biochem. **28**: 436-445, 1969.

Rufty, T.W., Jr., Kerr, P.S., Huber, S.C.: Characterization of diurnal changes in activities of enzymes involved in sucrose biosynthesis. - Plant Physiol. **73**: 428-433, 1983.

Sicher, R.C., Kremer, D.F.: Possible control of maize leaf sucrose-phosphate synthase activity by light modulation. - Plant Physiol. **79**: 695-698, 1985.

Stitt, M.: Fructose-2,6-bisphosphate as a regulatory metabolite in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **41**: 153-185, 1990.

Stitt, M., Huber, S.C., Kerr, P.: Control of photosynthetic sucrose formation. - In: Hatch, M.D., Boardman, N.K. (ed.): Biochemistry of Plants. Vol. 10. Pp. 327-409. Academic Press, New York 1987.

Stitt, M., Wilke, I., Feil, R., Heldt, H.W.: Coarse control of sucrose-phosphate synthase in leaves: alteration of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. - Planta **174**: 217-230, 1988.

Weiner, H., McMichael, R.W., Jr., Huber, S.C.: Identification of factors regulating the phosphorylation status of sucrose-phosphate synthase *in vivo*. - Plant Physiol. **99**: 1435-1442, 1992.

Worrell, A.C., Bruneau, J.M., Summerfelt, K., Boersig, M., Voelker, T.A.: Expression of a maize sucrose-phosphate synthase in tomato alters leaf carbohydrate partitioning. - Plant Cell **3**: 1121-1130, 1991.