Fructan synthesis is inhibited by phosphate in warm-grown, but not in cold-treated, excised barley leaves

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Summary

- The inhibition by phosphate of fructan accumulation has been investigated in warm-grown and cold-treated barley (*Hordeum vulgare* L.) plants.
- Detached leaves were incubated in water or phosphate for 24 h under lighting or in darkness. Fructosyltransferase, sucrose phosphate synthase (SPS) and cytosolic fructose- 1, 6 - bisphosphatase (FBPase) activities were subsequently analysed, as well as the content of carbohydrates, hexose-phosphates, phosphate, amino acids and protein.
- In warm-grown leaves, phosphate decreased fructan accumulation and total carbon in carbohydrates and did not affect protein contents. Phosphate increased hexose-phosphates, phosphate and amino acids. Fructosyltransferase and FBPase activities were not affected by phosphate feeding, while SPS activity was inhibited by phosphate in incubations both in light and in darkness. In cold-treated leaves, which prior to incubation had higher SPS activities than warm-grown leaves, phosphate had no inhibitory effect on fructan accumulation, carbohydrate content or total carbon in carbohydrates. SPS and FBPase activities were unaffected by phosphate.
- The results indicate that phosphate decreases fructan accumulation through an inhibition of SPS whenever this activity is not high prior to a rise in phosphate content.

Keywords: barley (*Hordeum vulgare*), fructan, fructosyltransferase, phosphate, sucrose phosphate synthase, regulation

Introduction

Fructans are fructose polymers present as reserve carbohydrates in cereals, grasses and many other plants. Fructans are synthesized from sucrose, a threshold concentration of which is required for fructan production (Pontis, 1970; Pollock *et al*., 2003) and the induction of gene expression and enzyme activity for fructan synthesis (Wagner *et al*., 1986; Pollock & Cairns, 1991). Fructan accumulation is enhanced under conditions such as drought (de Roover *et al*., 2000), low temperatures (Tognetti *et al*., 1990; Pérez *et al*., 2001), or nitrogen deficiency (Wang & Tillberg, 1996; Morcuende *et al.* 2004), which also induce sucrose:sucrose fructosyltransferase or sucrose:fructan 6-fructosyltransferase activities (Wang & Tillberg, 1996; van den Ende *al*., 1999; de Roover *et al*., 2000; Wang *et al*., 2000; Morcuende *et al.* 2004). The only reports of a phosphate effect on fructan contents are those of Russell (1938) and Wang and Tillberg (1997), who found an increase in fructan upon phosphate starvation. Phosphate did not affect the activities of fructan synthesis enzymes, and an effect on sucrose synthesis was suggested (Wang and Tillberg, 1997).

Phosphate is an inhibitor of two enzymes responsible for sucrose synthesis, FBPase and SPS (Huber & Huber 1996; Strand *et al.* 2000). SPS is allosterically activated by glucose-6-phosphate (G6P) and is inhibited by phosphate in some plant species; G6P is also an inhibitor of SPSkinase, while phosphate inhibits SPS protein phosphatase, which points to a role of these effectors in SPS activation through dephosphorylation (Huber & Huber 1996). In addition to its role in SPS modulation, low phosphate levels inhibit fructose-6-phosphate, 2 kinase and decrease Fructose-2, 6-bisphosphate (Fru2,6bisP) content, thus enhancing FBPase activity (Strand *et al.* 2000). The role of phosphate in fructan synthesis through the modulation of sucrose synthesis enzymes has not yet been established.

This work reports certain experiments that were carried out to investigate the mechanisms of phosphate regulation of fructan synthesis. Since low temperatures increase the activity of sucrose and fructan synthesis enzymes, we compared the effects of phosphate in illuminated excised leaves of plants grown at warm temperatures and also in cold-treated leaves. Phosphate effects were also assessed with the low carbohydrate levels of darkened leaves. Since export from detached leaves is inhibited (Krapp et al., 1991), the accumulation of carbohydrates accurately reflects the relative fluxes into each component, which is not always the case with attached leaves. Fructosyltransferase, SPS and FBPase activities were measured to identify possible sites of regulation by phosphate. The content of carbohydrates, amino acids and protein were analysed to assess the changes in carbon allocation induced by phosphate feeding.

Materials and Methods

Plant material

Seeds of barley (*Hordeum vulgare* L. cv. Clarine) were sown in 2 l pots (25 seeds per pot) containing perlite; these were placed in a growth room with 350 µmol m^{-2} s^{-1} photon flux density (fluorescent plus incandescent) in a 16 h photoperiod, 22 ºC day/16 ºC night temperatures and 70 % relative humidity. The plants received water and a nutrient solution (Morcuende *et al*., 2004). In a second experiment carried out during winter, the pots were moved into an unheated glasshouse when three leaves had emerged. The glasshouse had maximum/minimum temperatures of 17.8 ºC/ 1.8 ºC for 10 days, followed by 10.0 ºC/-0.4 ºC for 12 days. Irradiance was above 500 µmol m^{-2} s^{-1} in the central hours of the day. The plants were left in the glasshouse for 24 days, until the fifth leaf was fully expanded.

Treatment of excised leaves

The youngest fully expanded leaf in a shoot was cut with a sharp scalpel, and the cut end immediately placed in water for 30 min and then in 5 cm high Petri dishes with the cut end dipped in water or the test solutions through slots in the covers, as described (Morcuende *et al*., 2004). In a first experiment, leaves developed in the growth room were incubated in water or 5 mM K_2HPO_4/ KH_2PO4 (1.7:1 w/w); in the second experiment, cold-treated leaves were incubated in water or 0.5, 2 or 5 mM phosphate. The incubations were carried out for 24 h under continuous light or darkness under the conditions of the growth room described above. Treatments were arranged at random in four blocks in the first experiment, and three in the second, each consisting of four Petri dishes (two leaves per dish) per treatment. At the end of the incubation period, the

leaves were cut above the dish cover and rapidly transferred *in situ* to liquid nitrogen and stored at -80 ºC until analysed.

Analysis of compounds and enzyme activities

The pool of metabolically accessible phosphate was determined by feeding leaves (two additional leaves per treatment and block) with 200 mM glucose in water or phosphate (at the concentrations used during incubations) for 30 min following the 24 h incubations, and analysing the increases in glucose-6 phosphate (G6P) and fructose-6 phosphate (F6P) (Strand *et al*., 1999), as described below.

The content of fructans, other carbohydrates and amino acids, and fructosyltransferase activity were determined in subsamples of frozen leaves as in Morcuende *et al*. (2004), and hexose-phosphates as in Pérez *et al*. (2001). For the extraction of total proteins, frozen leaf material (100 mg fresh weight) was ground to a fine powder using a pestle and mortar pre-cooled with liquid nitrogen and homogenised with 1 ml of ice-cold extraction buffer containing 50 mM Tricine buffer (pH 8), 75 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 5 mM ε-aminocaproic acid, 2 mM benzamidine, 0.14 % (v/v) β-mercaptoethanol, and 1 mM phenylmethylsulfonylfluoride (PMSF). An aliquot of the homogenate was used to precipitate proteins with one volume of cold acetone containing 0.07 % (v/v) β-mercaptoethanol and 20 % (w/v) trichloroacetic acid (TCA) in 100% acetone. After incubation of the mixture at –20 ºC for 2 h, the extract was centrifuged at 20000 g for 15 min at 4 ºC to pellet the precipitated proteins and the supernatant was removed. The pellet was washed three times with ice-cold 100% acetone with 0.07 % (v/v) β-mercaptoethanol until it was completely white. The rest of the acetone from the pellet was removed by heating in a drying chamber at 40 ºC for 30 minutes. Proteins were solubilized with 1 ml of 50 mM

Tris-HCl buffer (pH 8) containing 100 mM sucrose, 3.5 % (w/v) SDS, 1 mM EDTA, and 0.07 % (v/v) β-mercaptoethanol by incubation at room temperature and shaking for 20 min and further incubation at 70 ºC for another 20 min. After cooling to room temperature, the samples were centrifuged at 20000 g for 15 min and the supernatant was decanted. The total protein content in the supernatant was determined spectrophotometrically at 750 nm using the Lowry *et al.* (1951) method with slight modifications (Peterson, 1977), using bovine serum albumin as a standard.

For cytosolic FBPase assays, frozen leaf subsamples were ground to a fine powder using a mortar and pestle pre-cooled with liquid nitrogen and were extracted in ice-cold 50 mM HEPES-KOH (pH 7.5) buffer containing 12 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM benzamidine, 1 mM h0aminocaproic acid, 1 mM DTT, 0.1 % Triton X-100, 1 mM PMSF and 1% PVPP. A spectrophotometric method coupled to NADP reduction was used for the FBPase assays (Pérez *et al*., 2001). SPS was assayed by measuring either the sucrose plus sucrose-6-phosphate or the UDP produced from fructose-6-phosphate and UDP-glucose. Replicate analyses were performed with high substrate and effector concentrations to measure both the activated and inactivated forms of SPS (V_a activity, Trevanion *et al*. 2004) or with low concentrations of these in the presence of the inhibitor phosphate to measure only the activated form (V_b activity, Trevanion *et al*. 2004); SPS activation was estimated as the V_b/V_a ratio. First, the widely used procedure described for spinach (Huber *et al*., 1989) was followed. However, very low V_a SPS was obtained in leaves incubated with phosphate. This was overcome with the method of Trevanion *et al*. (2004), optimised for wheat, in which enzyme activity involves greater changes in affinity for UDP-glucose and reduced sensitivity to inhibition by phosphate than in spinach. However, the desalting step with Sephadex G25 (Amersham Biosciences Europe GmbH, Barcelona, Spain) failed to

sufficiently remove the sugars, probably fructans, giving high blanks and variability in the anthrone test. Thus, numerous assays were required to obtain reliable results. The alternative assay of UDP was unsuitable due to low recovery of UDP, probably because of high UDP phosphatase activity (Trevanion *et al.* 2004). An alternative method was used in which the leaves were extracted in ice-cold 50 mM HEPES-KOH (pH 7.5) buffer containing 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM benzamidine, 5 mM h0aminocaproic acid, 5 mM DTT, 10 μM leupeptine, 0.5 % BSA, 0.1 % Triton X-100, 1 mM PMSF and 2% PVPP. After centrifugation at 17000 g at 5 ºC for 5 min, the undesalted supernatants were made 40 % PEG-6000, allowed to stand on ice for 40 min, and then centrifuged at 17000 g at 5 ºC for 5 min. The precipitate was then resuspended in the extraction buffer without PVPP and assayed with the concentrations of substrate, effectors and phosphate described by Trevanion *et al.* (2004). The PEG precipitation step substantially decreased the amount of interfering sugars so that reliable results could be obtained. The SPS assay was validated with tests of linearity of the enzyme activity with respect to time and amount of leaf extract.

Statistical analysis

The analyses of variance for a randomised block design experiment were performed with the GenStat 6.2 statistical package. From these analyses, the least significant differences ($P < 0.05$) among treatments were derived; these are shown in the figures.

Results

Fructan production is repressed by phosphate in warm grown leaves

The effect of phosphate on fructan biosynthesis was examined in detached illuminated leaves during 24h in the growth room conditions. Large amounts of carbohydrates were accumulated at the end of the incubation period (compare leaves prior to and after incubations, Fig. 1A), showing that synthesis was very active. As for rates of carbohydrate synthesis, in other studies on excised leaves (Cairns et al. 2002; Morcuende et al. 2004) sucrose builds up for about 7 h and subsequently undergoes small changes until completion of the 24 h incubation, while fructans dramatically increase after 7 h incubations. The glucose-fructose ratios increased during incubations (1.57, 4.55 and 2.55, for leaves prior to incubation and leaves in water and phosphate after incubation, respectively), probably reflecting the fructan synthetic activity, which would incorporate fructose to the fructan pool, releasing free glucose from sucrose (Morcuende *et al.*, 2004).

Compared with water, phosphate decreased fructan concentrations in warm grown leaves (Fig. 1A). Phosphate also decreased glucose, fructose and sucrose contents. The sum of total carbon in the carbohydrates analysed was 2.77 and 2.13 mmol g^{-1} f. wt, lsd 0.215, for leaves in water and phosphate, respectively. The overall data indicate that phosphate decreased the accumulation of carbohydrates. Moreover, the fructan/starch ratio, which may be indicative of carbon partitioning, decreased with phosphate (data not shown).

Phosphate supply strongly increased hexose-phosphate and phosphate content, and decreased the G6P/total phosphate ratio (Fig. 1B), showing a build-up of intermediates for the synthesis of sucrose, the substrate for fructan production, and an improved balance between SPS effectors. Total phosphate is shown in Fig. 1B, rather than

cytosolic phosphate, whose analysis (see Materials and Methods) was unsuccessful either because the uptake of the solutions was low after 24 h incubations of the leaves, or because 30 min was insufficient time. Amino acid concentrations were higher with phosphate than with water, suggesting a shift in carbon partitioning towards nitrogen compounds, and total protein contents did not vary significantly in response to incubations with phosphate (Fig. 1A).

Phosphate also decreased fructan and hexose concentrations in excised leaves incubated for 24 h in darkness, and affected the hexose-phosphate and phosphate content, as well as the fructan/starch ratios, as described for leaves incubated under light (data not shown).

Phosphate has no effect on fructosyltransferase, but inhibits SPS activity in warm grown leaves

In order to ascertain whether phosphate decreased fructan content by inhibiting the fructan synthesis enzymes, the sucrose-dependent fructosyltransferase activity was measured following light and dark incubations of excised leaves (Fig. 2A). Phosphate did not decrease this activity. The possibility that fructan synthesis might have decreased through the inhibition of substrate synthesis was explored by assaying SPS and cytosolic FBPase activities before and after the incubations. During the incubations of leaves under light, V_a SPS activity rose by 1.5-twofold (Fig. 2B). Phosphate decreased V_a and thus V_b SPS compared with water. The inhibitory effect of phosphate on V_a SPS was confirmed with the low sucrose levels, non-inductive of fructosyltransferase activity, in leaves incubated in darkness for 24 h (Fig 2B). In contrast, cytosolic FBPase activity in light-incubated leaves was not inhibited by phosphate (Fig. 2C).

Phosphate does not inhibit fructan biosynthesis in cold treated excised leaves

The effects of phosphate on fructan synthesis were further examined in another experiment with plants growing for an extended period at low temperatures, which are known to increase the activities of sucrose and fructan synthesis enzymes, and the concentrations of phosphorylated intermediates, fructans and other carbohydrates (Hurry *et al*., 2000; Pérez *et al.*, 2001). In order to verify whether the effect of phosphate was concentration-dependent, leaves were incubated with several levels of the anion.

Except for a smaller concentration of starch, leaf levels of fructans, other carbohydrates, amino acids, hexose-phosphates and phosphate were higher in cold-treated than in warm-grown leaves at the start of incubation (compare Fig. 3A with Fig. 1A), which is consistent with previous results at low temperatures (see above). During the 24 h incubations of cold-treated leaves, a moderate increase in fructose, sucrose and amino acids occurred, together with a strong accumulation of glucose, starch and fructan (Fig. 3A), which shows an active synthesis of carbohydrates in detached leaves; in contrast, there was little change in hexose-phosphate and protein levels (Fig. 3B).

Incubation of cold-treated leaves with phosphate had no significant effect on fructan content compared with water-incubated leaves (Fig. 3A), in contrast to the inhibition by phosphate of fructan accumulation found in warm-grown leaves. Phosphate increased sucrose and starch, did not affect fructose and decreased glucose content in cold-treated leaves. These effects on sucrose and glucose increased with phosphate concentration in the solution. Thus, in contrast to the experiment with warm-grown leaves, total carbon in carbohydrates did not decrease but instead increased with incubation in phosphate (2.24 and 2.76 mmol g^{-1} f. wt, lsd 0.277, for leaves in water and 5 mM phosphate,

respectively), indicating that the accumulation of carbohydrates was enhanced by phosphate. The increase in starch content after phosphate feeding conflicts with the triose-P translocator-mediated model for starch accumulation. However, it is consistent with the operation of an alternative, ill-characterized, regulatory system (Cairns *et al.*, 2002). As in warm-grown leaves, the fructan/starch ratio decreased with phosphate feeding, more so as the concentration of phosphate increased (data not shown).

Phosphate also increased the concentration of G6P, while the G6P/total phosphate ratio – and thus the balance between SPS effectors - decreased (Fig. 3B). Incubations with 5 mM phosphate increased amino acid concentrations relative to water, while the lower phosphate levels had no effect (Fig. 3 A). Phosphate did not affect total protein content significantly compared with water (Fig. 3A).

SPS activity is not affected by phosphate in cold treated excised leaves

Since phosphate did not decrease fructan accumulation as in the preceding experiment, in which an inhibition of SPS was associated with this decrease, the response of SPS activity to the supply of the highest concentration (5 mM) of phosphate was analysed in this experiment. In attached leaves prior to incubation, V_a SPS activities were higher in cold-treated than in warm-grown leaves (0.52 vs. 0.30 µmol g^{-1} f. wt min^{-1}, respectively). During the 24 h incubations, SPS activity showed small changes, in contrast to warm-grown leaves (compare Fig. 4A with Fig. 2B). V_a and V_b SPS activities did not change significantly with phosphate compared to water (Fig 4A). There was no effect of phosphate on FBPase activity (Fig 4B).

Discussion

Fructan production is repressed by phosphate in warm grown leaves

Our results show that high leaf phosphate contents can decrease fructan accumulation, in agreement with previous reports (Wang and Tillberg, 1997), although other factors may render fructan synthesis insensitive to phosphate. The absence of differences in fructosyltransferase activity between phosphate- and water-fed leaves indicates that phosphate does not decrease the accumulation of fructans by inhibiting the enzymes of its synthesis from sucrose, in agreement with Wang and Tillberg (1997), in spite of the involvement of protein kinases and phosphatases in the induction of sucrose-dependent fructosyltransferase activity (Martinez Nöel *et al.*, 2001). This contrasts with the recently reported role of nitrate as a negative signal for fructosyltransferase(s) (Morcuende *et al.* 2004). It has been suggested (Wang and Tillberg, 1997) that an inhibition by phosphate of the enzymes of sucrose synthesis could decrease fructan levels. The decreased leaf sucrose content observed after supplying phosphate to warm-grown plants (Fig. 1A) suggests a restricted synthesis of the substrate for fructan production. Moreover, here we show that the decreased accumulation of fructans by phosphate in warm-grown plants is associated with an inhibition of V_a SPS, but not cytosolic FBPase activity. After 24 h incubations under light, there was a large increase in SPS activity (Fig. 2B), as observed previously (Trevanion *et al*., 2004); phosphate restricted this increase with time. A relationship between phosphate and the amount of SPS protein can be deduced from the fact that a phosphate-accumulating Arabidopsis mutant has been reported to have less SPS protein, while a phosphate deficient mutant had threefold higher SPS protein levels than the wild type (Hurry *et al.*, 2000). In contrast to the decrease in V_a, SPS activation was not significantly affected by phosphate feeding as compared to water in our experiments.

Barley leaves, like wheat, store large amounts of sucrose that do not lead to the SPS inactivation found in starch-storing species, such as spinach (Trevanion *et al*., 2004), an inactivation which is likely due to changes in the phosphorylation status of the enzyme (Lunn and Furbank, 1999). Further research may show whether other enzymes involved in sucrose synthesis and subjected to phosphate regulation, such as UDPglucose pyrophosphorylase (Cieresko *et al.*, 2001), limit fructan accumulation in phosphate-fed leaves. As for the specificity of phosphate effects on sucrose and fructan synthesis, an experiment comparing phosphate and sulphate feeding to excised leaves (unpublished results) suggests a general role for inorganic anions (Huber *et al*., 1994) in fructan synthesis inhibition, although some variation in the mechanisms involved may exist. The decrease in total carbon in carbohydrates when anions were supplied to warm-grown leaves suggests a decrease in photosynthesis (Huber *et al*., 1994) as one of these mechanisms.

Phosphate does not inhibit fructan biosynthesis in cold-treated excised leaves

In contrast to leaves from plants developed in the growth room, cold-treated leaves did not undergo decreases in fructan levels nor inhibition of SPS in response to phosphate. This remarkable difference between the experiments could be due to variations in the factors modulating sucrose synthesis. Prior to incubation, cold-treated leaves had higher levels of hexose, sucrose, fructan and hexose-phosphate than warm-grown leaves, in agreement with earlier studies (Hurry *et al*., 2000; Pérez *et al*., 2001); phosphate levels were also higher, as observed in cold-developed leaves (Strand *et al.*, 1999). Although an estimation of total phosphate in leaves may not necessarily represent the cytosolic phosphate pool (Strand *et al.*, 1999), it has been shown that phosphate feeding increases cytosolic phosphate, although less than vacuolar phosphate

(Bligny *et al.*, 1990), and low temperatures also increase cytosolic phosphate (Strand *et al.* 1999). The G6P/total phosphate ratio, which can be used to estimate the balance between SPS effectors, was higher in warm-grown than in cold-treated leaves incubated in water; it was lower with phosphate than with water with both leaf sources, and was similar for phosphate-fed leaves from both environments. Thus, the G6P/phosphate ratio would seem less suitable for sucrose synthesis in cold-treated than in warm-grown leaves; the increases in SPS activity (compare Figs. 2B and 4A) and in sucrose (Figs. 1A and 3A) after the 24 h incubations under light were actually lower in the former than in the latter. However, SPS activity in cold-treated leaves was already high before the incubations and was enough to sustain the observed increase in sucrose, fructan and hexoses during incubations (details not shown), in such a way that sucrose and fructan accumulation did not undergo inhibition by phosphate. As with warm-grown leaves, there was no significant decrease in SPS activation with phosphate compared with water, in contrast to phosphate-feeding experiments with spinach (Huber *et al*., 1994). Future research on SPS gene expression and protein content will be required to identify the underlying mechanism for the difference between warm-grown and cold-treated leaves.

In conclusion, phosphate limits fructan accumulation in warm-grown leaves because it restricts V_a SPS and not because of decreased activation of this enzyme. In leaves with high SPS activity, a rise in phosphate levels does not decrease SPS activity or fructan synthesis. Where fructan accumulation is restricted, there is a decrease in total carbon in carbohydrates, pointing to an inhibitory effect of phosphate on photosynthesis.

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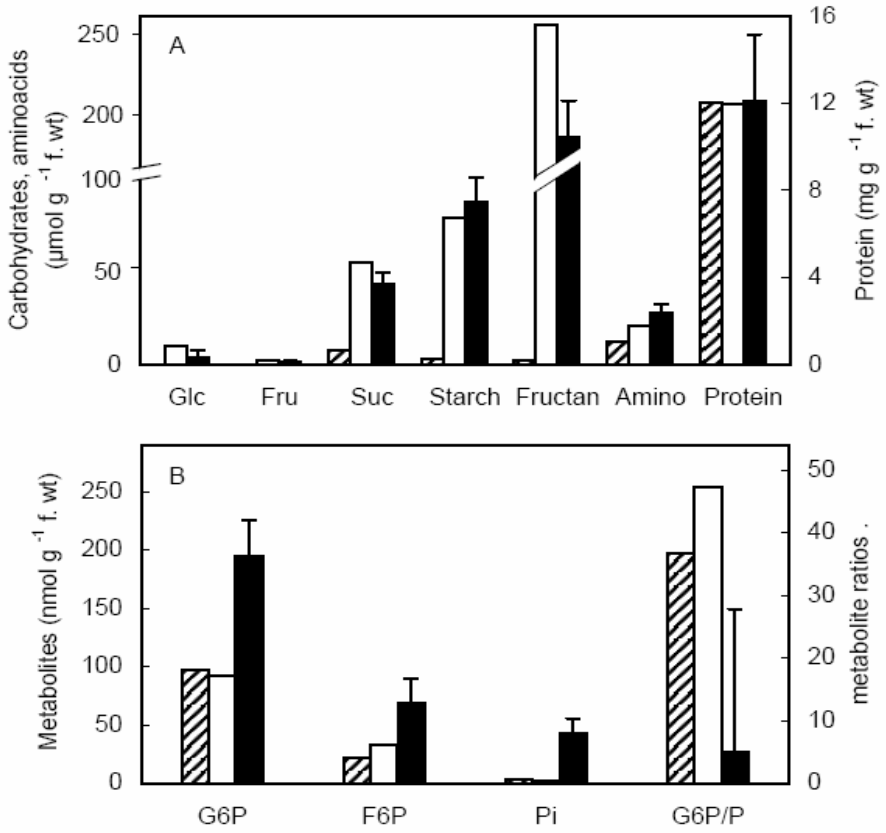
Legends to figures

Fig. 1. Concentration of carbohydrates, amino acids (μmol g^{-1} f. wt) and protein (mg g^{-1} f. wt) (A), hexose-phosphates and phosphate (B) in warm-grown barley (*Hordeum vulgare* L.) leaves incubated under 350 μmol m^{-2} s^{-1} light intensity in water (open columns) or 5 mM phosphate (black columns) for 24 h. Hatched columns, attached leaves under 350 μmol m^{-2} s^{-1} irradiance at the start of incubations. Values are means of four replicates. Vertical bars in this and successive Figures represent least significant differences ($P < 0.05$) between means of incubation solutions.

Fig. 2. Fructosyltransferase activity with sucrose as substrate (A), V_b (open columns) and V_a (black columns) SPS activity and activation (tinted columns) (B) and cytosolic FBPase activity (C) in warm-grown barley (*Hordeum vulgare* L.) leaves prior to (Control), and after 24 h incubations in water or 5 mM phosphate (P) under light as described in Fig. 1, or in darkness (right y-axis in panel A).

Fig. 3. Concentration of carbohydrates, amino acids (μmol g^{-1} f. wt) and protein (mg g^{-1} f. wt) (A) and of hexose-phosphates and phosphate (B) in cold-treated barley (*Hordeum vulgare* L.) leaves prior to (hatched columns), and after 24 h incubations under 350 μmol m^{-2} s^{-1} irradiance in water (□), 0.5 mM phosphate (■), 2 mM phosphate (▩),or 5mM phosphate (▨). Values are means of three replicates.

Fig. 4. V_b (open columns) and V_a (black columns) SPS activities and activation (tinted columns) (A) and cytosolic FBPase activity (B) in cold-treated barley (*Hordeum vulgare* L.) leaves prior to (Control) and after 24 h incubations under 350 μmol m^{-2} s^{-1} irradiance in water or 5 mM phosphate (P). Values are means of three replicates.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F6P
Pi
G6P/P

Fig. 1

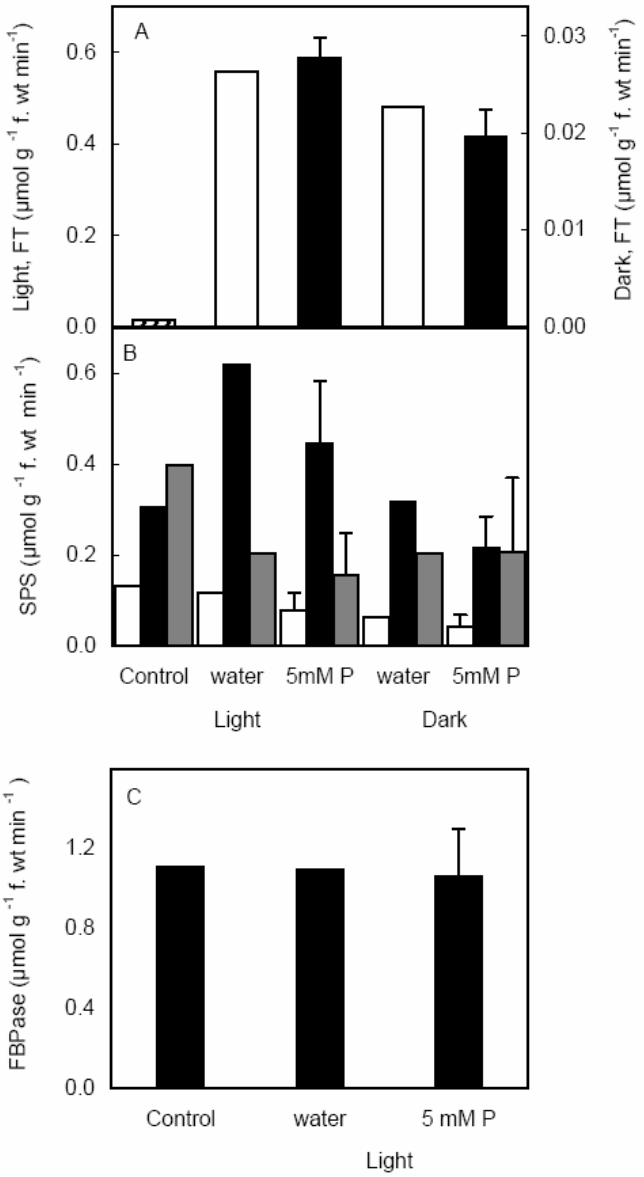
A
B
C
Light, FT (µmol g -1 f. wt min-1)
Dark, FT (µmol g-1 f. wt min-1)
SPS (µmol g -1 f. wt min -1)
FBPase (µmol g -1 f. wt min -1)
0.0
0.2
0.4
0.6
0.00
0.01
0.02
0.03
0.8
1.2
Control
water
5mM P
water
5mM P
Light
Dark
Control
water
5 mM P
Light

Fig. 2

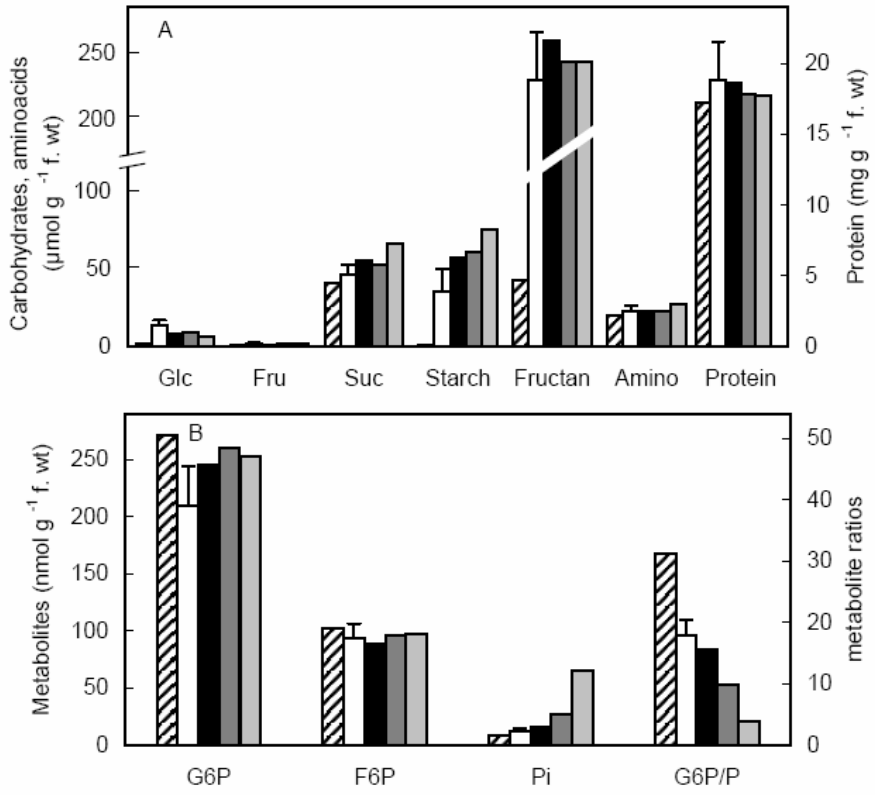
A
Carbohydrates, aminoacids (µmol g -1 f. wt)
Protein (mg g -1 f. wt)
0
50
100
200
250
0
5
10
15
20
Glc
Fru
Suc
Starch
Fructan
Amino
Protein
B
Metabolites (nmol g -1 f. wt)
metabolite ratios
0
50
100
150
200
250
0
10
20
30
40
50
G6P
F6P
Pi
G6P/P

Fig. 3

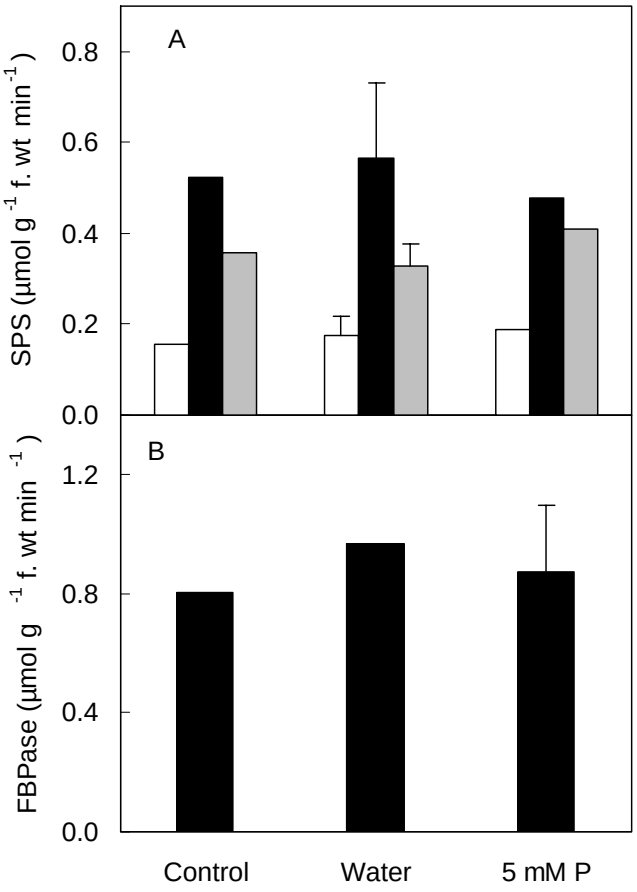
A
B
SPS (µmol g -1 f. wt min-1)
FBPase (µmol g -1 f. wt min -1)
0.0
0.2
0.4
0.6
0.8
1.2
Control
Water
5 mM P

Fig. 4