Response of the carbohydrate metabolism and fructose-2,6-bisphosphate to environmental changes : effect of different light treatments

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Response of the carbohydrate metabolism and fructose-2,6-bisphosphate to environmental changes. Effect of different light treatments

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Abstract

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Important and rapid variations of Fru-2,6-P_2 were observed during light-on and light-off transitions in both spinach and mustard: Fru-2,6-P_2 underwent tremendous variations showing an 80% decrease within 13 minutes in both plants. In mustard cotyledons, during both day and night periods, Fru-2,6-P_2 showed a decrease during the first hour, followed by a regular 240% increase. In spinach, the same behavior of Fru-2,6-P_2 was observed, except that once the concentration of the sugar had doubled, it remained constant. In contrast to mustard, spinach Fru-2,6-P_2 presented a much higher level during the night than during the light period. In 3 day old germinating oat, red light illumination for 5 min was accompanied by a 48 h halt of elongation and by a 180% increase of Fru-2,6-P_2 20 min after irradiation, followed by the total disappearance of this sugar 80 minutes after light treatment.

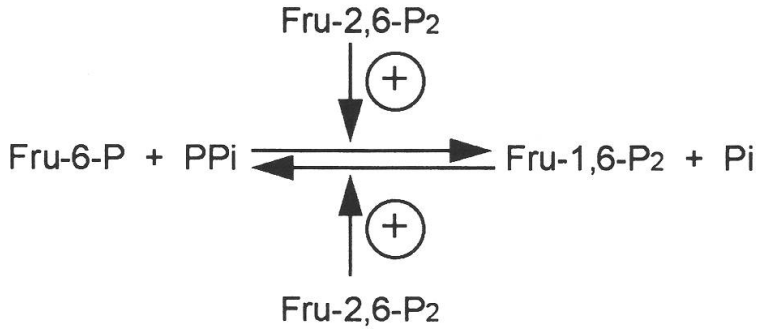
The present work shows the highly dynamic capacity of the regulatory sugar Fru-2,6-P_2 in plants with respect to short, mid and long term light treatments. This indicates an important cellular messenger role of Fru-2,6-P_2 in the regulation of carbohydrate metabolism in response to light changes.

Key words: Spinacia oleracea, Sinapis alba, Avena sativa, Fructose-2,6-bisphosphate, carbohydrate metabolism, photoperiodism.

Introduction

During the 80's, it was shown that Fru-2,6-P_2 plays a central role in sugar metabolism as it regulates the activities of several enzymes: those connected to glycolysis are generally activated, while those involved in gluconeogenesis are inhibited (Huber, 1986). Interactions of this molecule with its target enzymes could thus readjust the fluxes and metabolite pool in the cytosol (Stitt, 1990). The role of Fru-2,6-P_2 has become particularly important in plants since it regulates the activities of key enzymes: PPi-dependent fruc-

tose-phosphate 1-phosphotransferase (PFP) (Sabularse and Anderson, 1981 van Schaftingen et al., 1982) and cytoplasmic fructose-1,6-bisphosphatase (cFBPase) (Stitt, 1990; Kruger and Beevers, 1984). PFP, is catalyzing the following interconversion:



This enzyme has a high affinity for its activator with a K_a generally situated between 1.56 nM and 50 nM (van Schaftingen et al., 1982; Bertagnolli et al., 1986; Degli Agosti et al., 1992; van Praag, 1996). It is present in the cytosol of plant cells as either α_2 or β_2 homodimers or as a $\alpha_2\ \beta_2$ heterotetramer. The α-subunit is suggested to play a regulatory role by binding Fru-2,6-P_2, while the β-subunit contains the catalytic activity (Carlisle et al., 1990). Recent computer models of the tetrameric $\alpha_2\ \beta_2$ form of PFP confirm that Fru-2,6-P_2 binds between the α- and β-subunits, with interactions between the 6′-phosphoryl group and the oxygen of the furanose ring with the α-chain (van Praag, 1996).

Fru-2,6-P_2 is generally present in micromolar quantities in plant tissues with concentrations ranging between 1 and 30 µM in spinach (Cséké et al., 1982; Stitt, 1990), maize (Stitt, 1990) or wheat leaves (Stitt, 1990) as well as in citrus juice cell protoplasts (van Praag, 1996). The level of this phosphorylated sugar further seems to follow an oscillating pattern in plant tissues (Stitt, 1990; Paz et al., 1985). This is rather interesting as Fru-2,6-P_2 generally correlates negatively with the accumulation of sucrose in photosynthetic leaves (Stitt, 1990). Such accumulation of sucrose during the day was shown in primary leaves and petioles of spinach plants (Steiner, 1968 a), followed by a drop of sugar content during the night (dark period) (Steiner, 1968 b). Interestingly, a regular increase in the concentrations of glucose and fructose but not of sucrose was observed in plants exposed to a prolongation of the light period (transfer treatment) (Degli Agosti et al., 1990). The same response was also noticed in etiolated mustard hypocotyls exposed to five minutes of red light. Indeed, the levels of free hexoses increased continuously in mustard hypocotyls from the moment they were shortly illuminated with red light (Steiner, 1968 a, b), but it was concluded that it was an indirect consequence of irradiation.

In an effort to understand the mechanism that induce the accumulation of glucose and fructose in plant cells exposed to a sudden modification of the light regime, we decided to examine the possible involvement of Fru-2,6-P_2 in spinach or mustard leaves and in etiolated oat germs.

Materials and methods

Plant material

Spinach (*Spinacia oleraceae* L. cv. Nobel) plants, grown on soil, and mustard seeds (*Sinapis alba* L.), grown on vermiculite, were cultivated in a growth chamber with standard irradiation conditions

(20 $W m^{-2}$) provided by Sylvania fluorescent lamps ("day-light" F40T12, 40 W). The temperature was maintained at 20 ± 0.5 °C and the relative humidity varied between $70 \pm 5\%$ during the day and $50 \pm 5\%$ at night. Plants were grown in a short day photoperiod: 8 hours light and 16 hours darkness. The transfer experiments were done with 30 day old spinach plants and 10 day old mustard plants.

Oat seeds (*Avena sativa* L. cv. Garry) were planted on wet vermiculite in closed boxes, which were kept in complete darkness for a number of days. Temperature of germination was 21 ± 1 °C.

Extraction of Fru-2,6-P_2

Extraction was done at 0 °C. The tissue samples were transferred into a cold grinding glass, 1 ml of cold NaOH 0.5 M and 3 ml of chloroform were added before extraction. After grinding extensively, the extract was vortexed and centrifuged at $4000 \times g$ during 5 min. The aqueous phase was collected and stored at − 80 °C. Recovery of added Fru-2,6-P_2 in control samples was 98.8%.

Measurement of Fru-2,6-P_2

The possible interaction of other plant constituents can modify the activity of PFP and its response to Fru-2,6-P_2. It is thus essential that activation of Fru-2,6-P_2 is calibrated by comparison with an internal standard curve using pure Fru-2,6-P_2 plus the extracts. The standard assay medium (1 ml) contained: 50 mM Hepes-NaOH, pH 7.5; 2 mM Mg-acetate; 1 mM Fru-6-P; 10 mM Fru-2,6-P_2; 0.15 mMN NADH; 0.45 U aldolase (EC 4.1.2.13); 5 U triose-phosphate isomerase (EC 5.3.1.1); 1.7 U glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) and 0.01 U of potato PFP. The mixture was preincubated in a water bath for 5 min at 25 °C and the reaction was initiated upon addition of PPi (final concentration: 0.5 mM) and 10 to 20 µl of sample to the cuvette. Oxidation of NADH was followed in a spectrometer at 340 nm.

Results

1. Fru-2,6-P_2: fast and medium term response to changeable photoperiods

The levels of Fru-2,6-P_2 were followed in mustard and spinach leaves when light was turned on and off (Fig. 1). Rapid sampling revealed that the level of this sugar showed tremendous changes in concentration within a short period of time. Indeed, some time before the light was switched on in the morning, the level of Fru-2,6-P_2 showed a 2.5 fold increase in mustard cotyledons, followed by a rapid disappearance at the moment the light was turned on (Fig. 1 A). Similar results were obtained for spinach leaves (Fig. 1 C). In both cases, the responses to light transition were extremely fast: a 77% decrease of Fru-2,6-P_2 within 9 min in mustard cotyledons and a 80% decrease within 13 min in spinach leaves.

After the photoperiod, when the light was switched off again, comparable fluctuations were noted in mustard (Fig. 1 B) and in spinach leaves (Fig. 1 D). In mustard tissue, the level of Fru-2,6-P_2 increased instantly within 3–4 minutes after nightfall, followed by a sharp decline and recovery. A similar increase after darkness was observed in spinach leaves, however, the level of Fru-2,6-P_2 stayed high when plants remained in darkness (Fig. 1 D).

Fru-2,6-P_2 concentrations were further followed over longer periods of time, during the light and dark phases of the short day photoperiod. In mustard cotyledons (Fig. 2), a decrease of the sugar diphosphate content occurs during the first hour of light, followed by a regular increase of 233% during the next 5 hours. Two hours before nightfall, the level of Fru-2,6-P_2 started to decline (Fig. 2).

During the dark period, the pattern of Fru-2,6-P_2 is rather similar to that obtained during the light period. During the first 60 min a strong decrease is observed, after which it increases with peak-concentrations every two hours in the case of mustard cotyledons.

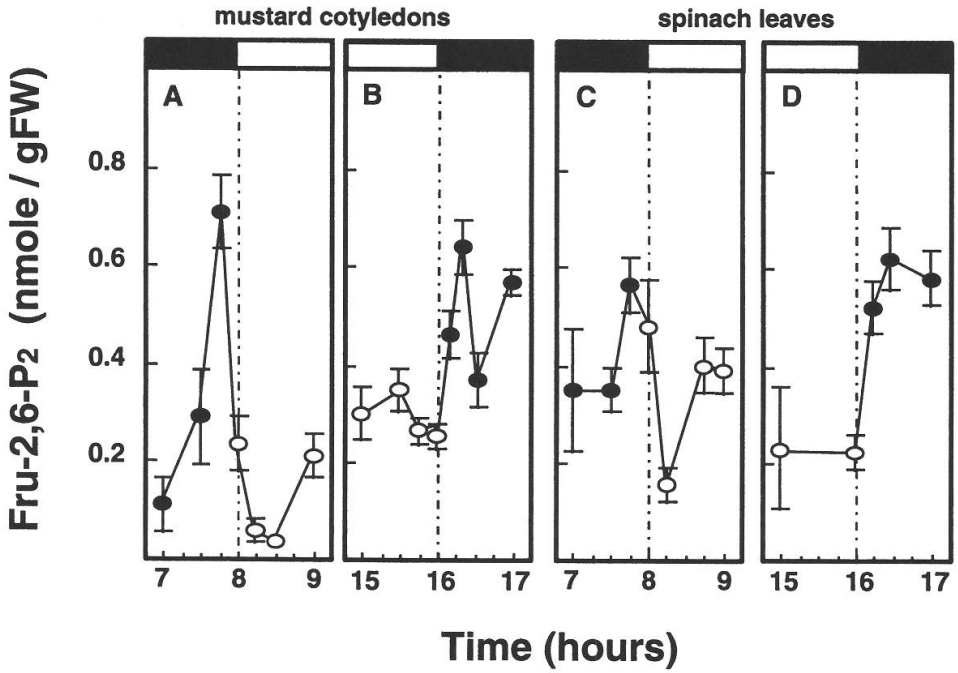


Fig. 1. Changes in the level of Fru-2,6-P_2 in mustard cotyledons (A + B) and spinach leaves (C + D) kept at a short day photoperiod during the dark/light (A + C) and light/dark (B + D) transition periods.

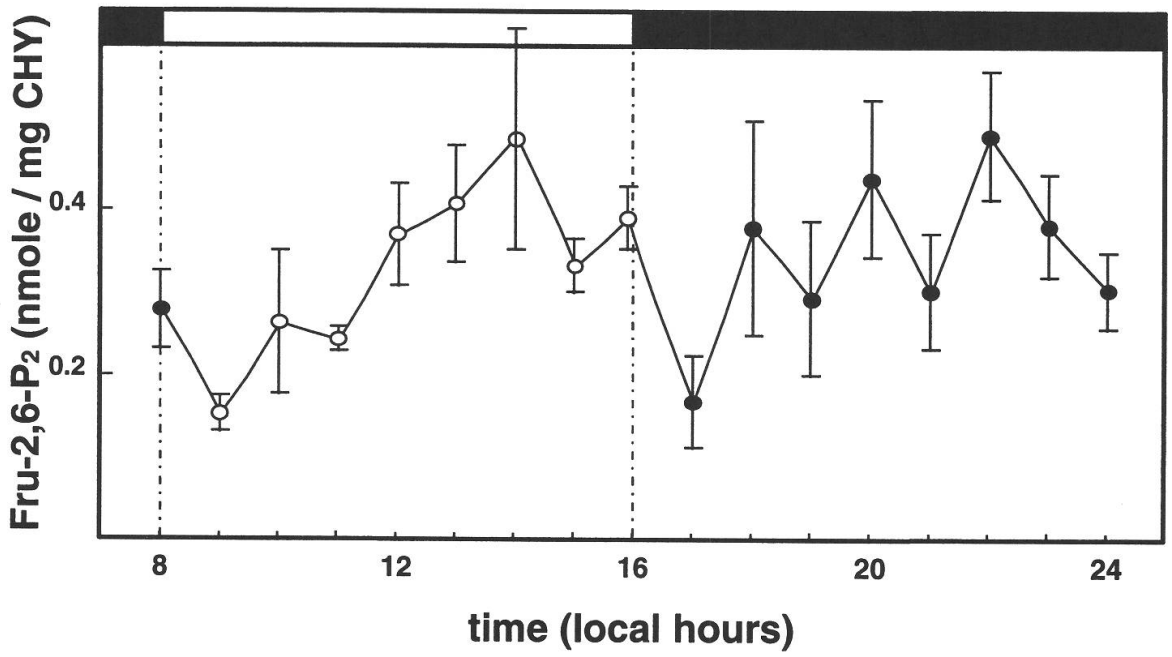


Fig. 2. Variation in the content of Fru-2,6-P_2 in mustard cotyledons during a short day photoperiod (8 : 16 L : D) in light (o) and dark (•).

The concentrations of Fru-2,6-P_2 are comparable to those reached during the day (0.5 nmole/mg chlorophyll (CHY)), representing a 240% increase.

Similar results were obtained with spinach leaves (Fig. 3). However, the level of Fru-2,6-P_2 reached during the night is almost twice that reached after 4 hours of illumination (Fig. 3).

2. Germinating oat: growth, effect of light and concentrations of Fru-2,6-P_2

It was shown that day light changes induce a rapid response in Fru-2,6-P_2 levels in leaves, but could 5 min of red light illumination influence the concentration of this sugar in 3 day old etiolated oat? The mean concentration of Fru-2,6-P_2 in the germs exposed to green light before irradiation is equal to 0.34 ± 0.02 nmole/gFW. Twenty minutes after irradiation, the level of Fru-2,6-P_2 increased by 180% (Fig. 4), followed by a steady decrease till it cannot be detected anymore after 80 minutes.

The growth of the irradiated germs was further followed to check if a 5 min irradiation was long enough to induce morphological modifications in 3 day old etiolated germs. At this age, the epicotyl is about 10 mm long (Fig. 5C). Twenty four hours later, clear differences in growth were observed. Indeed the germs grown in complete darkness and green light were 2.5 times longer than those exposed to 5 min of light (Fig. 5). However, growth of the irradiated samples resumed after 48 hour as in those who had remained in darkness (Fig. 5).

The concentrations of Fru-2,6-P_2 has also been examined in growing germs to study long term fluctuations (Fig. 6). The concentration of Fru-2,6-P_2 was followed from the moment that the germ appears on the 3rd day up to the 6th day and it showed large differences: up to 0.65 nmole/gFW on the 4th and 6th day and 0.18 nmole/gFW the 5th day (Fig. 6). At this stage the roots are well developed (Fig. 5C). After the fast drop of

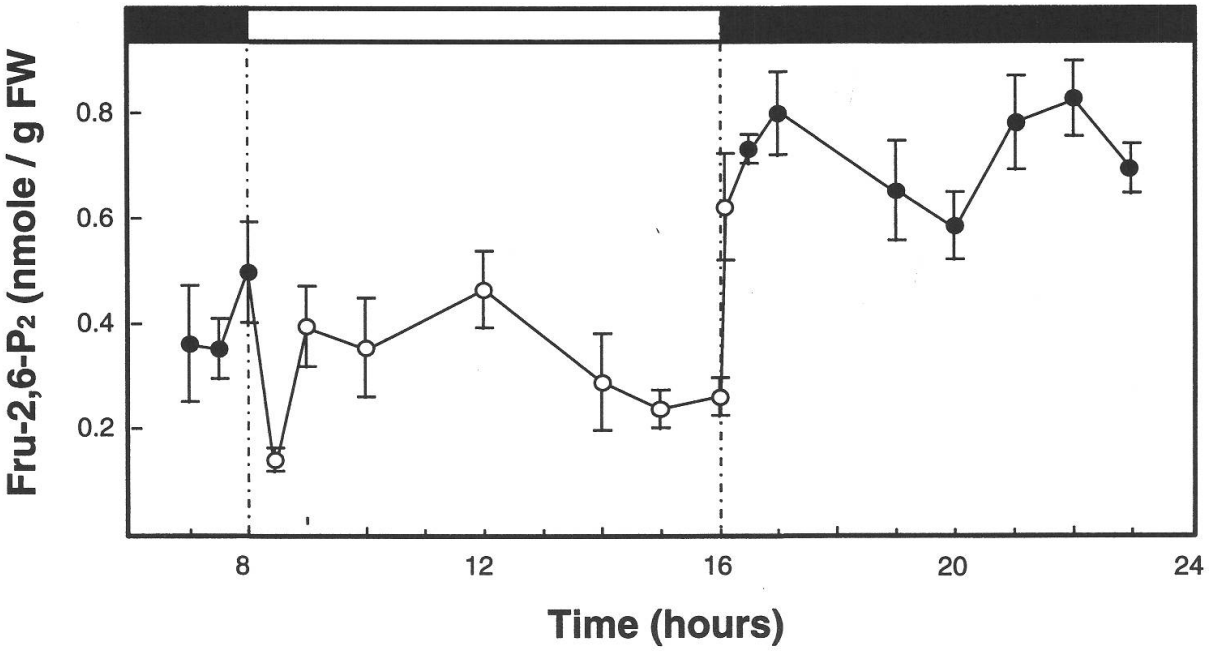


Fig. 3. Variation in the content of Fru-2,6-P_2 in spinach leaves during a short day photoperiod (8:16 L:D) in light (o) and dark (●).

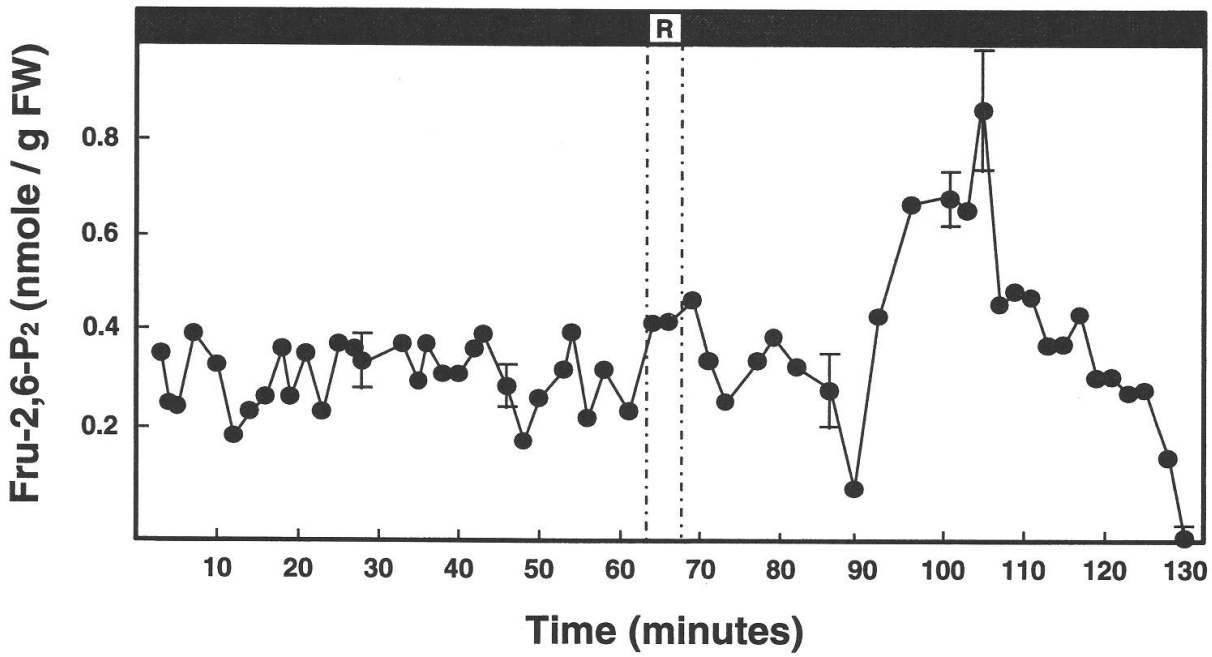


Fig. 4. Variation in the content of Fru-2,6-P_2 in germinating oat (3 day old) grown in complete darkness (●), after receiving 5 min of red light on the 3rd day.

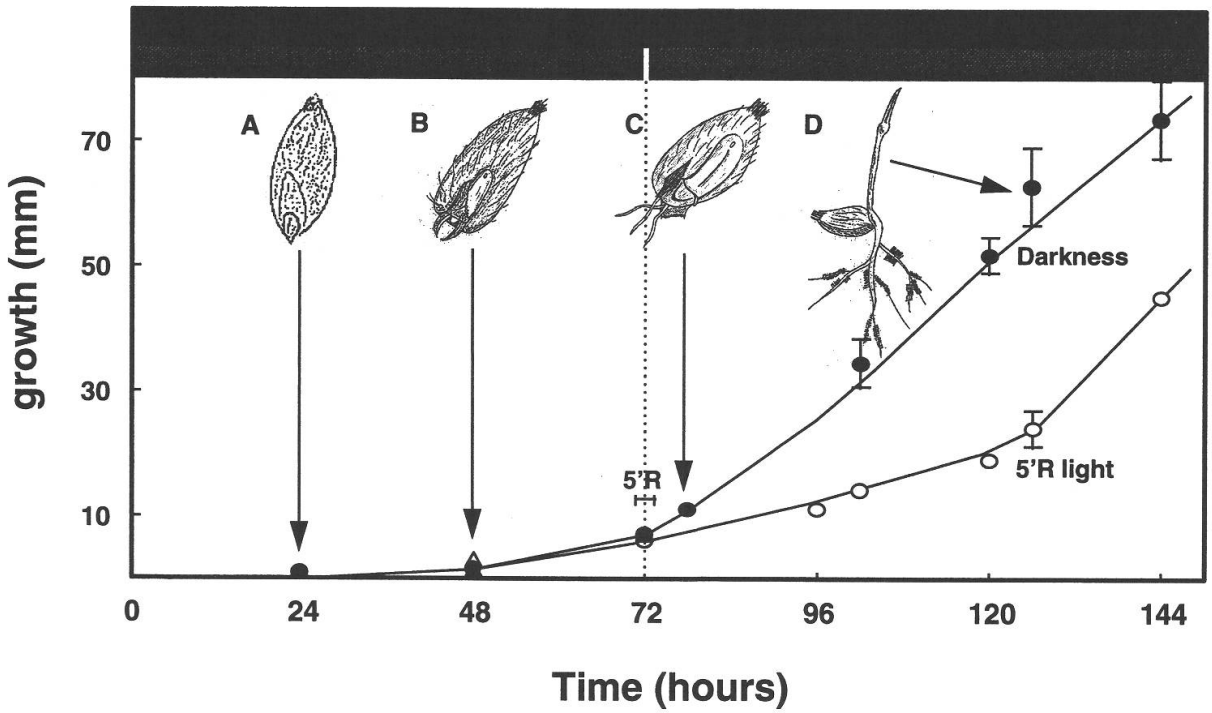


Fig. 5. Growth of germinating oat kept in complete darkness (●) and after irradiation with red light during 5 min on the 3rd day (○). A: oat seed after a 24 hour period of soaking. B: appearance of the germ, 48 hours after sowing. C: growth of the oat germ and appearance of the root at the age of 72 hours. D: the developing germs separates into hypocotyl and primary leaf. The roots are well developed and present absorbing hairs.

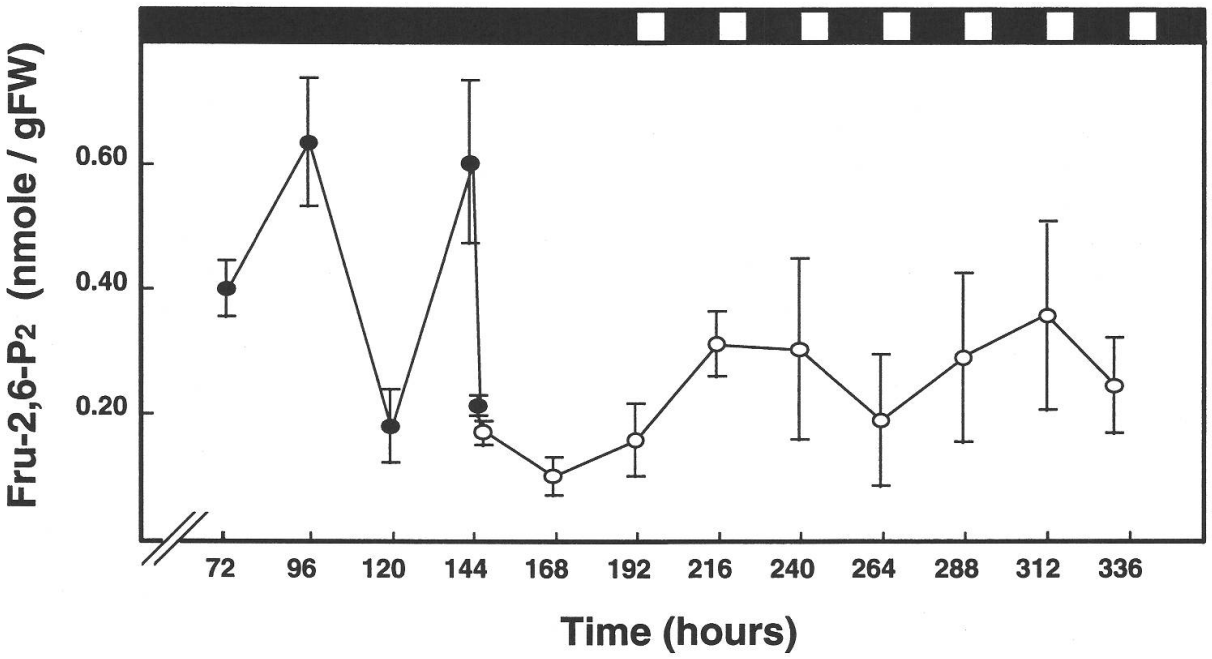


Fig. 6. Long term evolution of Fru-2,6-P_2 content in germinating oat grown in complete darkness (●) and moved to a short day photoperiod at the age of 6 days (○).

Fru-2,6-P_2 observed in the beginning of the 6th day, the etiolated oat germs were placed in a short day-light photoperiod. Till the 8th day, the level of Fru-2,6-P_2 remains low, with an average value of 0.15 nmole/gFW. After the elevation observed on the 9th day, Fru-2,6-P_2 seems to reach a plateau-concentration (Fig. 6).

Discussion

In this work, it was shown that light is a very important environmental factor as oat germs, spinach leaves or mustard primary leaves modify their endogenous amount of Fru-2,6-P_2 in its presence.

Levels of Fru-2,6-P_2 in leaves during the day and the night photoperiod

Measurement of Fru-2,6-P_2 content in spinach and mustard leaves show that the levels vary according to the day and night phases. While it took 6 hours to reach the maximum level of Fru-2,6-P_2 during the light period, it took only 5 hours to reach this value in the dark period in mustard leaves. In spinach leaves, the level of Fru-2,6-P_2 was immediately much higher than those noticed during the light period. During the periods of light changes (light-on and light-off), the level of Fru-2,6-P_2 underwent tremendous variations within minutes. Such rapid fluctuations were also observed in other plant tissues (Baysdorfer and Robinson, 1985; Fahrendorfer et al., 1987; Sicher et al., 1986; Stitt et al., 1984; Usuda et al., 1987). The results obtained for the Fru-2,6-P_2 concentrations after light-off (Fig. 1 B and 2) seem contradictory. From different experiments with short sampling intervals (data not shown) it became clear that the level of Fru-2,6-P_2 regularly decreased to a threshold concentration after a maximum was reached during

the photoperiod. Generally this minimal concentration was attained before the moment of light-off (see e.g. Fig. 3). However, if light-off occurs before the minimal threshold is reached (see Fig. 2, minimum of Fru-2,6-P_2 around 17 h), the short term fluctuations are present while the mid-term adaptation is delayed.

These results, combined with thos obtained previously for hexose, sucrose and starch in the same plants (Degli Agosti and Greppin, 1987; Degli Agosti et al., 1989) seem to suggest that carbohydrate metabolism is regulated by endogenous factors that are synchronized with the photoperiod. One of those factors could be related to the accumulation of triose-phosphates in the cytosol of plant cells. These sugars are exported from the chloroplast into the cytosol of photosynthetic tissues. Semiquantitative models were developed to understand the role of Fru-2,6-P_2 in the regulation of the glycolysis/gluconeogenesis (Stitt, 1990). It was found that triose-P needs to accumulate in the cytosol, until a "threshold" is reached to activate cFBPase. The sensitive response to rising triose-P is generated as the concentration of Fru-2,6-P_2 increases and that of Fru-2,6-P_2 decreases. The last compound is indeed known to regulate the activity of cytosolic FBPase, which catalyses the irreversible dephosphorylation of Fru-1,6-P_2 into Fru-6-P. The enzyme is totally inhibited in presence of micromolar concentrations of Fru-2,6-P_2. This value is nearby an order of magnitude higher than the activation constant of PFP. Assuming that the cytosol represents 4 to 10% of the cell volume, the higher levels of Fru-2,6-P_2 measured in oat germs, mustard or spinach leaves are thus high enough to activate PFP and completely inhibit cFBPase (van Schaftingen et al., 1982; Degli Agosti et al., 1992; van Praag, 1996; Stitt, 1990).

Mid and long term response of Fru-2,6-P_2 in germinating oat

It is known that red light affects numerous physiological and morphological processes in plants: e.g. a reduction of stem growth. During the post-germinative stages of oat seeds, an important regulator of carbohydrate metabolism, Fru-2,6-P_2, was present at high concentrations in oat germs kept dark. The high level of this constituent can be compared with those measured in previous experiments (Sicher and Bunce, 1987) and agrees with the needs of the growing plant: sugars stored in the seeds are transported to the growing tissues where they are metabolized through the glycolytic pathway. Glucose, fructose and triose-phosphates are produced and enable the biosynthesis of additional molecules needed for the growth process. Decreased levels of Fru-2,6-P_2 on the 5th day correspond to the stage were more than 60% of the starch stored in the seed has been exported to the growing tissues (Degli Agosti et al., 1991). This stage also shows already well developed roots, that should be able to take up the necessary minerals from the soil. Transition from sink tissue to source tissue can thus be expected to take place, enabling the etiolated germs to start gluconeogenesis, a metabolic pathway that can only be performed when the Fru-2,6-P_2 level is low.

The level of Fru-2,6-P_2 present in the germs that were placed in a SD photoperiod corresponds to those measured in mustard and spinach leaves. The greening of the coleoptile is accompanied by the development of chloroplasts. Once photosynthesis takes place, the chloroplasts start to export triose-phosphates to the cytosol and sucrose can be formed through gluconeogenesis.

The results obtained after exposing 3 day old etiolated germs to 3 min of red light show that the amount of Fru-2,6-P_2 is rapidly affected. Indeed, a sudden increase is noticed 20 min after irradiation, followed by total disappearance 60 min later. This dramatic effect of the Fru-2,6-P_2 content, accompanied by the increase of glucose and

fructose (Steiner, 1968 a, b) could possibly indicate that Fru-2,6-P_2 acts as an initial signal for further metabolic events that help the plant cell to reorganize itself in order to adapt to the new environmental conditions. The rapid fluctuations of Fru-2,6-P_2 could indeed be the signal that triggers the dissociation of PFP from its tetrameric glycolytic form into its gluconeogenic dimer. This hypothesis is strengthened by the fact that almost no increase of reducing sugars was observed in germs placed in continuous FR light after the R irradiation (Steiner, 1968 a, b).

The results further show that some specific parameters (time of the day, rate of sampling) need to be respected in experiments dealing with measurements of Fru-2,6-P_2 levels in plant tissues. Particularly in long term studies, it is important to collect the daily samples at exactly the same time of the day to be able to compare the evolution of the Fru-2,6-P_2 levels of this specific moment. Studies dealing with the Fru-2,6-P_2 levels during the light and night periods in plants will, however, only indicate the general tendency of the effector molecule, as it is shown in the present study that its concentrations can vary considerably in very short periods of time.

Resumé

Le Fru-2,6-P_2, régulateur du métabolisme des sucres, présente des modifications de sa concentration à court, moyen et long terme chez diverses plantes lors de variations lumineuses. Il fluctue de manière journalière dans les cotylédons de moutarde et les feuilles d'épinard. Lors des phases lumineuses, on observe une diminution durant les premières heures, suivie d'une augmentation régulière d'environ 240%. On observe également des fluctuations importantes et rapides au moment des transitions nuit/jour et jour/nuit chez ces deux plantes. L'irradiation par 5 min de lumière rouge sur des germes d'avoine étiolés modifie aussi bien la croissance que le niveau de Fru-2,6-P_2. Celui-ci augmente de 180% 20 min. après le traitement et diminue progressivement par la suite.

Les résultats obtenus montrent que le Fru-2,6-P_2 possède une remarquable capacité dynamique d'adaptation à court, moyen et long terme. Ces adaptations sont présentes aussi bien chez des dicotylédones (moutarde et épinard) que chez une monocotylédone (avoine). Ceci met en évidence le rôle important joué par le Fru-2,6-P_2en tant que messager cellulaire du métabolisme des sucres en répone à diverses variations lumineuses de l'environnement.

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