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RESPONSE OF FRUCTOSE-2,6-BISPHOSPHATE TO ENVIRONMENTAL CHANGES. EFFECT OF LOW TEMPERATURE IN WINTER AND SPRING WHEAT.

BY

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ABSTRACT

Response of fructose-2,6-bisphosphate to environmental changes. Effect of low temperature in winter and spring wheat - Three day old, etiolated, wheat seedlings were affected differently according to the duration of exposure to a non-freezing low temperature (5°C). Indeed, growth was only reduced after a 45 min or longer treatment. Germs exposed to a 120 min cold period were half the size 24 h after the cold treatment, in comparison to the room temperature (20°C) exposed seedlings. In respect with these previous results, the content of Fru-2,6-P2 was further analyzed in wheat germs. A ten min cold exposure did not induce any obvious modifications. A longer treatment (120 min, 5°C) induced important fluctuations in the concentration of Fru-2,6-P2. These were significantly larger than those observed in the control treatment. Such fluctuations were present in two winter wheat varieties (Fidel, Vienna) and, to a lesser extent, in one spring variety (Frisal). The present work shows the high dynamic capacity of changes of the regulatory sugar Fru-2,6-P2 in plants with respect to temperature changes.

Key-words: Wheat, *Triticum aestivum*, Fructose-2,6-bisphosphate, carbohydrate metabolism, non-freezing cold.

Abbreviations: ADP, adenosine diphosphate; ATP, Adenosine triphosphate; cFBPase, cytosolic fructose bisphosphatase; FW, fresh weight; Fru-6-P, fructose-6-phosphate; Fru-2,6-P2, Fructose-2,6-bisphosphate; Hepes, 4-(2-Hydroxyethyl)-piperazine-1-ethane sulfonic acid; NADH, nicotinamide adenine dinucleotide (reduced form); Pi, phosphate; PFK, ATP-dependent phosphofructokinase; PFP, pyrophosphate-dependent phosphofructokinase; PPi, pyrophosphate; SPS, sucrose phosphate synthase.

INTRODUCTION

The interconversion of Fru-6-P to Fru-1,6-P2 is a key reaction in the control of the carbohydrate metabolism. In plants, this reaction is catalyzed by three cytosolic enzymes: ATP-dependent phosphofructokinase (PFK), pyrophosphate dependent phosphofructokinase (PFP), and cytosolic fructose bisphosphatase (cFBPase). These enzymes are regulated by various metabolic intermediates and anionic molecules (VAN SCHAFTINGEN *et al.*, 1982; STITT, 1990; DEGLI AGOSTI *et al.*, 1992). The role played by PFP and cFBPase in the

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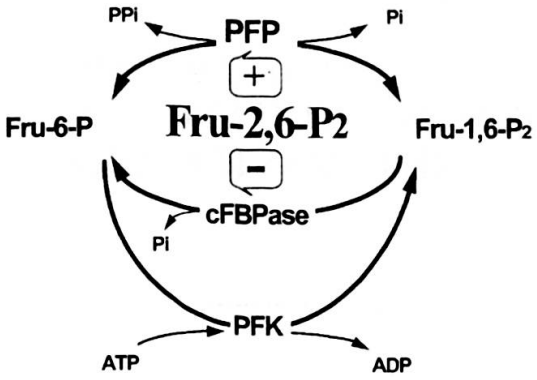
plant carbohydrate metabolism, however, only became evident when they were found to be regulated by a phosphate ester: Fru-2,6-P2 (VAN SCHAFTINGEN *et al.*, 1982; STITT, 1990):



The presence of Fru-2,6-P2 strongly enhances the activity of PFP, with an activation constant ranging in the nanomolar range (HUBER, 1986; STITT, 1990; DEGLI AGOSTI *et al.*, 1992; VAN PRAAG, 1997). It is furthermore suggested that Fru-2,6-P2 determines the state of aggregation of PFP in a dimer or a tetramer. Indeed, 3-D computer models of potato PFP suggest that the tetrameric form contains two different subunits: α and β and would be involved in the glycolytic reaction. The β subunit is responsible for catalysis, while the α subunit is involved in the binding of Fru-2,6-P2, thus in the regulation of the activity of PFP. The dimeric β2 form is not able to bind Fru-2,6-P2 and is suggested to catalyze the gluconeogenic reaction (CARLISLE *et al.*, 1990; VAN PRAAG, 1997).

Fru-2,6-P2 is located in the cytosolic compartment of plant cells, which represent 4 to 10% of the total cell volume. There, its concentration ranges between 1 and 7 μM in wheat (STITT, 1990) or in citrus juice cell protoplasts (VAN PRAAG, 1996) and between 0.1 and 0.5 nmole/gFW in spinach leaves or mustard cotyledons (VAN PRAAG *et al.*, 1997). Although fluctuations of the concentrations of this bisphophate sugar are observed upon modifications of the environmental conditions, e.g. light illumination, it also varies according to the plant's internal time keeping mechanism over a 24 h period and longer (VAN PRAAG *et al.*, 1997). Interestingly, the activities of some key enzymes of the carbohydrate metabolism, such as sucrose phosphate synthase (SPS) are also regulated by an internal (circadian) rhythm (LYONS-JOHNSON, 1996). A sudden exposure to a low non-freezing temperature is able to disrupt this pattern (LYONS-JOHNSON, 1996), leading to the alterations of important physiological and biological processes (FOWLER, 1992). On the long range, however, plants adapt to the new conditions: the activity of PFP remains fully operational (CLAASSEN *et al.*, 1991), carbohydrates are stored or fructan, a chain of fructose linked to sucrose molecules, is broken down (LEE, 1996).

In an attempt to understand the mechanism that command the readjustment of the carbohydrate metabolism in plants exposed to low temperatures, we decided to examine the possible involvement of Fru-2,6-P2 in etiolated wheat germs exposed to sudden temporary non-freezing cold.

MATERIALS AND METHODS

Plant material

For the growth experiments, seeds were sown in plastic boxes (10 x 10 x 10 cm) on wet paper, with the embryo directed upwards. The germinating seeds were kept at a temperature of 20 ± 1°C in complete darkness by wrapping the boxes with aluminium foil and stored in light-proof chambers until they were used and sampled in dim green safe-light. The length of about 35 coleoptiles was then measured with a ruler.

For the experiments dealing with the measurement of Fru-2,6-P2, thirty five wheat seeds (*Triticum aestivum* L., either the spring variety Frisal or the winter varieties Fidel and Vienna) were sown in the same way as described above, except that new Petri dishes (10 cm) were used instead of boxes. The cold treatment was started by placing the light-proof Petri in a refrigerator, whose temperature was set at at 5 ± 1°C. Temperature was monitored inside the Petri with a small electronic thermosensor. Samples, consisting of 10 (Fidel and Vienna) and 5 (var Frisal) coleoptiles were collected every 3 min and were immediately frozen in liquid N2. The opened boxes/dishes were immediately discarded once that the germs were sampled. Sampling was done in a dim green safe-light. Preliminary experiments have shown that dim green safe-light does not alter the Fru-2,6-P2 content (VAN PRAAG *et al.*, 1997) or gowth.

Extraction of Fru-2,6-P2

The different steps for the extraction of Fru-2,6-P2 were all carried out at 0°C. The samples were transferred from the liquid N2 into a cold grinding glass. The extraction media was composed of 1 ml of cold NaOH (0.5 M) and 3 ml of chloroform. After thorough grinding, the extract was vortexed and centrifuged at 4000 g during 5 min, after which the aqueous phase was collected for storage at -80°C. Recovery tests of commercial Fru-2,6-P2 in control samples was 98.8%.

Measurement of Fru-2,6-P2

It is essential that the activation of PFP by Fru-2,6-P2 is calibrated with an internal standard using pure Fru-2,6P-2 plus the extracts. The standard assay medium, as described previously (DEGLI AGOSTI *et al.*, 1992) (1 ml) contained: 50 mM Hepes-NaOH, pH 7.5; 2 mM Mg-acetate; 1 mM Fru-6-P; 10 mM Fru-6-P; 0.15 mM 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 the Fru-2,6-P2 extract into the cuvette. Oxidation of NADH was followed with a spectrophotometer at 340 nm.

RESULTS

The development of 3 day old winter wheat germs (var. Fidel) was followed to check if exposure to a non freezing cold (5°C) would affect the growth of coleoptiles. In

a first experiment, wheat seedlings were exposed to different periods of cold, ranging from t = 0 to 120 min and the eventual effect of temperature was measured 24 h later (Fig. 1). The control germs (t = 0 min) had an average length and standard deviation (S.D.) of 25.9 ± 5.6 mm. Interestingly this result is statistically (two-tailed Student t test, $P > 0.05$) not different from those observed after an exposure of t = 15 and 30 min (25.0 ± 4.5 mm and 23.2 ± 5.1 mm, respectively). The statistical evaluation of the obtained results indicate, however, that the average length of the germs becomes different after a 45 min cold exposure (17.3 ± 4.0 mm) and longer, as compared to the control ($P<0.01$).

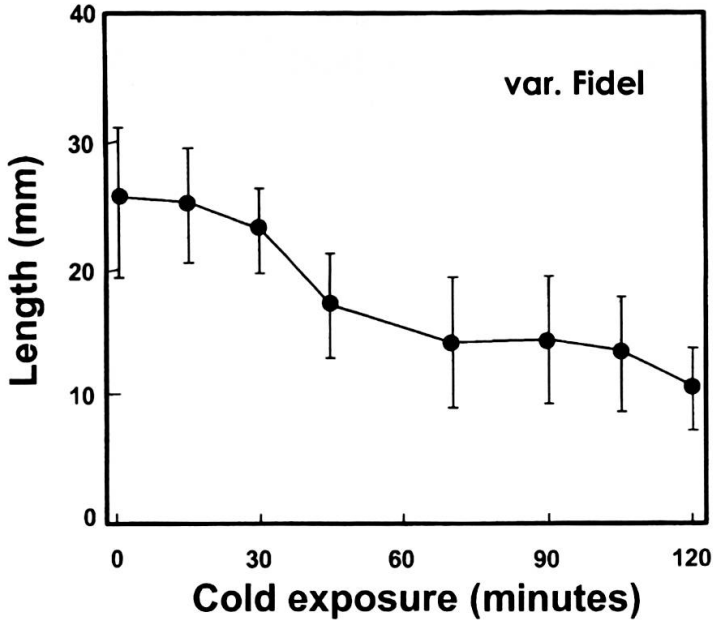


FIG. 1.

Length of dark kept winter wheat seedlings coleoptiles (var. Fidel) 24 hours after exposure to different times of non-freezing cold (5°C) on the 3rd day after sowing.

After a 120 min cold exposure, length of the germs was thus only half of those that remained at room temperature, 24 hours after the treatment (Fig. 1).

When growth was followed for several days in 3 day old control germs and in germs that were exposed to a 150 min cold, development was altered in the cold treated seedlings during the 2 days following the treatment (Fig. 2). This observation was confirmed by a statistical analysis (two tailed Student t test, $P << 0.001$). On the 3rd day after cold exposure, the average length's of the germ becomes statistically equal ($P > 0.01$).

These results obtained for the growth of winter wheat germs led us to analyze the level of Fru-2,6-P_2 in germs exposed to room temperature and to t = 10 and 120 min cold periods. Plants that were kept at room temperature showed no major variations in the concentration of Fru-2,6-P_2 (Fig. 3). As expected from the growth observations, a 10 min exposure to a low temperature also did not affect the level of Fru-2,6-P_2 in Fidel wheat germs (Fig. 3).

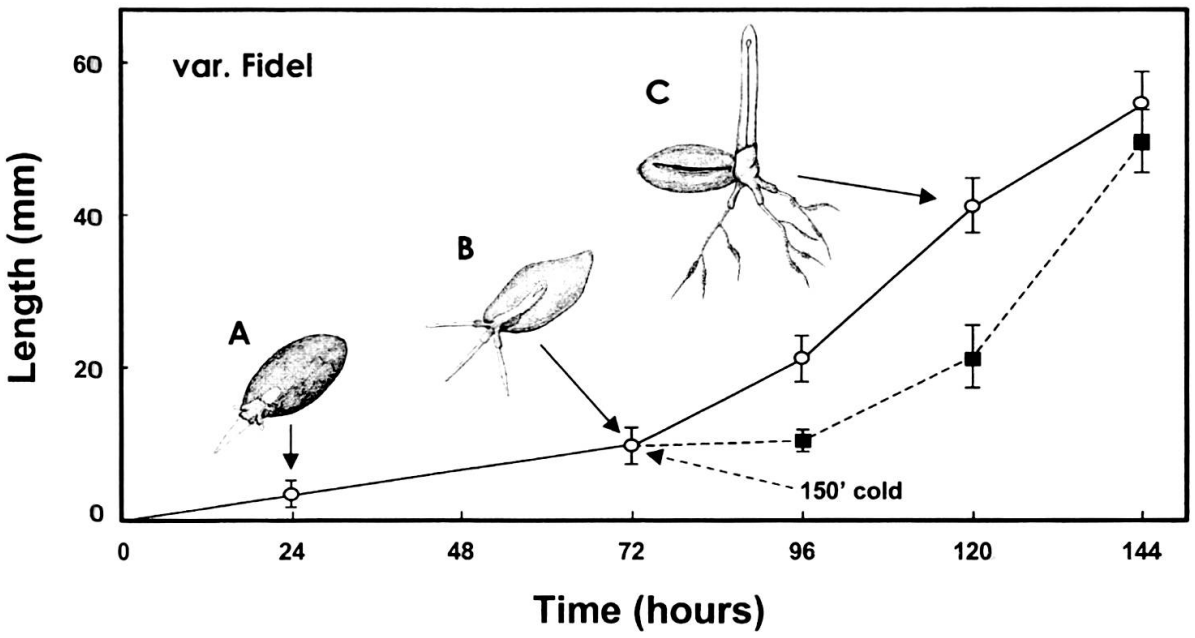


Fig. 2.

Comparison of the elongation of the dark kept winter wheat coleoptiles (var. Fidel) exposed to a room temperature (○) of 20°C and to a 120 min period of non-freezing cold (5°C) (■) on the 3rd day after sowing. A: wheat seed after a 24 hour period of soaking. B: appearance of the coleoptile, 48 hours after sowing. C: growth of the wheat seedling and appearance of the root at the age of 120 hours. The roots are well developed and possess absorbing hairs.

When 3 day old wheat germs (var. Fidel) were exposed to longer periods of non-freezing cold, a different response on the level of Fru-2,6-P2 was observed. Indeed, the level of Fru-2,6-P2 during the first 30 min of cold treatment remained in the same range as that observed at room temperature. After this lag period important fluctuations in the level of Fru-2,6-P2 appeared with a first 116% increase in the level of this sugar followed by its return to a mean level. A second rapid increase of 180% was observed within the next 20 min (Fig. 3).

When other winter (var. Vienna) and a spring (var. Frisal) wheat varieties were used, rapid fluctuations of Fru-2,6-P2 were also observed (Fig. 4) in both varieties, but to a lesser extent in the spring wheat (Fig. 4). Indeed, in the latter, variations in the level of Fru 2,6-P2 are clearly present, but without the corresponding high peaks observed with winter varieties. In order to confirm the visual observations of Fig. 3 and 4, a statistical variance test was applied, by pooling separately data obtained before and after the temperature treatment, respectively. The variance was statistically equal before and after a 10 minute cold treatment. In the long term exposure experiments, however, the variance was statistically different after cold exposure for each variety (Tab. I).

DISCUSSION

In this work, it was shown that cold is a determinant factor of the environment affecting the growth rate in wheat seedlings. Corresponding readjustments of carbohydrate metabolism were observed with the endogenous concentrations of Fru-2,6-P2

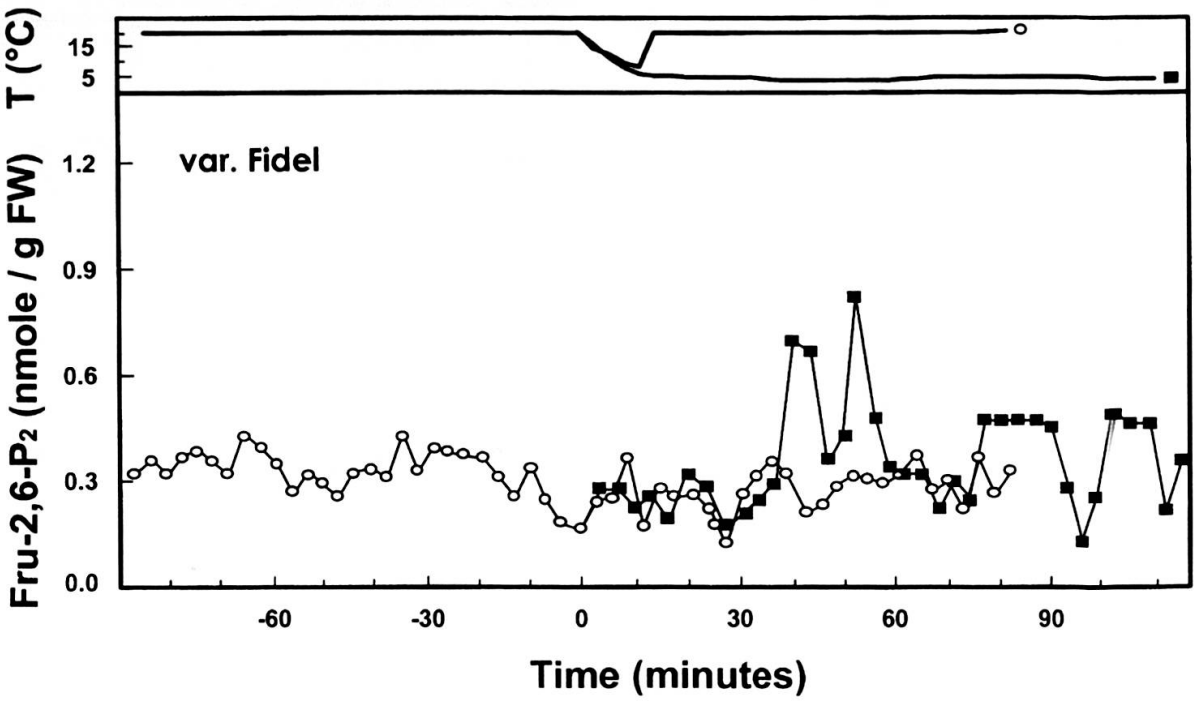


FIG. 3.

Variations in the level of Fru-2,6-P2 in dark kept wheat seedlings (coleoptiles), var. Fidel at room temperature (20°C) (○) followed by either a 10 min cold (5°C) period (○) or longer (■). Time zero is exactly 3 days after sowing.

TAB. I.

F-test for Fru-2,6-P2 contents in the coleoptiles of different wheat varieties exposed to either room temperature (20 ± 1°C) or to cold (5 ± 1°C). Data is from the results presented in Figs. 3 and 4. P is the probability level for the F-ratio occurring by chance.

		Fru-2,6-P2 variance		F-Ratio	P
Group 1	Group 2	Group 1	Group 2		
		nmole / gFW	nmole / gFW		
Fidel room	Fidel 10'cold	0.065	0.062	0.952	0.804
Fidel room	Fidel 120' cold	0.065	0.137	2.100	0.0001
Vienna room	Vienna 120'cold	0.082	0.237	2.912	<<0.0001
Frisal room	Frisal 120'cold	0.050	0.114	2.295	<<0.0001

in both winter and spring wheat. The decreased growth rate could be related with the diversion of the carbohydrate content in order to induce cold hardiness in wheat. Indeed, this process is known to begin in winter wheat plants when the temperature falls bellow 9°C (LEE, 1996). From our results it is also clear that a short cold treatment does not affect the level of Fru-2,6-P2. One reason could be that 10 min is too short to decrease the temperature well inside the tissue of the germ, which is not the case when the seedling is expo-

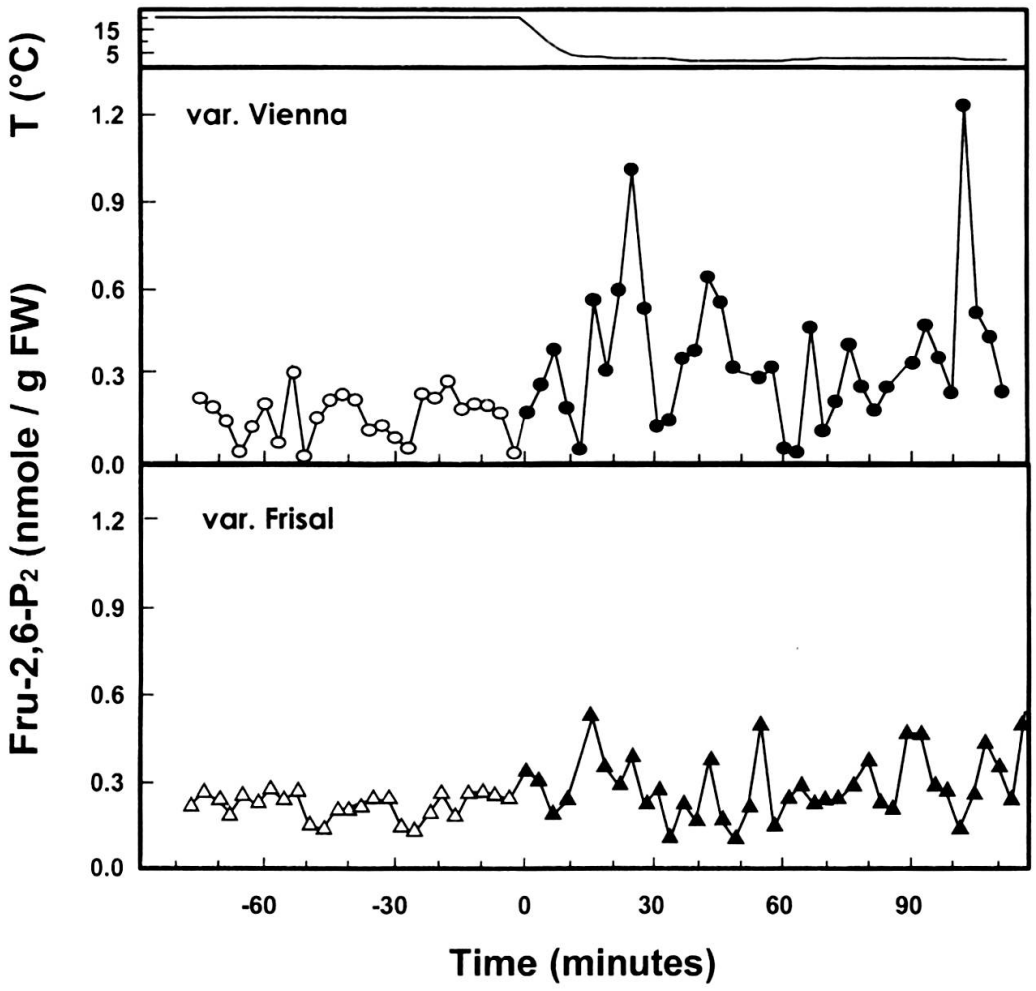


FIG. 4.

Variations in the level of Fru-2,6-P2 at room temperature (20°C) in dark kept wheat seedlings (coleoptiles), winter var. Vienna (○) and spring var. Frisal (Δ) and during a 120 min (●) in Vienna and in Frisal (▲) exposure to a non-freezing cold (4°C). Time zero is exactly 3 days after sowing.

sed for a longer period of non-freezing cold temperatures. Indeed, in the latter, fluctuations of the levels of Fru-2,6-P2 were induced, after a lag period of at least 20 min after transfer to the new environment. These variations are close to those observed in germinating oat exposed to red-light (VAN PRAAG *et al.*, 1997), except that the Fru-2,6-P2 level continued to fluctuate after a rapid initial increase in the cold exposed winter wheat germs, and in a more limited way in spring wheat.

Prolonged cold exposure is known to affect the activities of some key enzymes of the carbohydrate metabolism: cFBPase and SPS. The activity of potato PFP seemingly lacks cold susceptibility (CLAASSEN *et al.*, 1991), on the contrary to cFBPase, an enzyme inhibited by Fru-2,6-P2 and by low temperature in a reversible way (WEEDEN & BUCHANAN, 1983). This would indicate that the dephosphorylation of Fru-1,6-P2 is not catalyzed by cFBPase, but rather by PFP when a plant is exposed to a temperature of 5°C. The activity of SPS is also inhibited by low temperature, which affects the transcription of the

phosphatase gene and causes a delay in the circadian activity of this enzyme in chilling-sensitive plants such as tomatoes, soybean or corn (LYONS-JOHNSON, 1996). The fact that both cFBPase and SPS are affected by cold is interesting. Their activities are often linked to the plant's process of sucrose synthesis (HUBER, 1986) and the fast fluctuations of Fru-2,6-P2 observed in wheat germs during a long cold exposure could be involved in the regulation of their respective activities. PFP, however, was shown to possess a high activity for the gluconeogenic reaction in various plant tissues, activity that is generally less dependent on the presence of Fru-2,6-P2, as compared in the glycolytic reaction (VAN SCHAFTINGEN *et al.*, 1982; VAN PRAAG, 1997). The hypothesis that PFP rather than cFBPase is involved in the reorganization of the sugar metabolism is further supported by the fact that an accumulation of hexose-phosphates precedes the rise of the sucrose level in potato tubers exposed to cold (POLLOCK & AP REES, 1975). Fru-2,6-P2 would thus act as a signal that regulates the activity and/or the structural form of PFP in the cytosol, in order to accumulate carbohydrates of which some are known to act as cryoprotectants in plant tissues. This suggestion is supported by the fact that in barley and rye, half of the plant's glucose is used to prevent cell destruction by moving it to the apoplast (space between the cells) (LEE, 1996). The fluctuations of Fru-2,6-P2 could thus act as a message ordering the plant to reorganize the carbohydrate pool in order to protect itself in the new environmental conditions.

Moreover, the differences observed between the winter and spring varieties of wheat make it tempting to suggest that a correlation exist between the response of Fru-2,6-P2 and the variety of wheat, further studies are however necessary to validate the present suggestion. These results nevertheless once more emphasize the potential important role played by Fru-2,6-P2 in the reorganization of the carbohydrate metabolism in order to adapt to new environmental conditions (VAN PRAAG *et al.*, 1997).

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RÉSUMÉ

RÉPONSE DU FRUCTOSE-2,6-BISPHOSPHATE À DES VARIATIONS DE L'ENVIRONNEMENT. EFFETS D'UNE BASSE TEMPÉRATURE CHEZ LE BLÉ D'HIVER ET DE PRINTEMPS

La croissance de germes étiolés de blé âgés de 3 jours est modifiée différemment selon la durée d'un traitement au froid (5°C). Celle-ci est réduite de manière significative après une durée d'au moins 45 min de froid. Les germes exposés à 120 min de froid ont la moitié de la taille des témoins maintenus à température ambiante (20°C) 24 h après le traitement. La teneur en Fru-2,6-P2 des germes de blé a été analysée en relation avec ces résultats. Dix minutes de froid ne provoquent aucune modification évidente. Un

traitement plus long (120 min, 5°C) induit en revanche d'importantes fluctuations du Fru-2,6-P2. Celles-ci sont significativement plus grandes que celles mesurées dans les témoins. De telles fluctuations ont été observées chez deux variétés de blé d'hiver (Fidel et Vienna) et, dans une moindre mesure, chez une variété de printemps (Frisal). Ce travail montre que le Fru-2,6-P2 possède un très grande capacité dynamique chez les plantes vis-à-vis des changements de température.

Mots-clés: Blé, *Triticum aestivum*, Fructose-2,6-bisphosphate, métabolisme des sucres, basse température.

REFERENCES

CARLISLE, S., S.D. BLAKELEY, S.M. HEMMINGEN, S.J. TREVANION, T. HIYOSHI, N.K. KRUGER, C. CSÉKE, N.F. WEEDEN, B.B. BUCHANAN & K. UYEDA. 1990. A special fructose bisphosphate functions as a cytoplasmic regulatory metabolite in green leaves. *Proc. Natl. Acad. Sci. USA*, 79: 4322-4326.

CLAASSEN, P.A.M., M.A.W. BUDDE, H.J. DE RUYTER, M.H. VAN CALKE & A. VAN ES. 1991. Potential role of pyrophosphate: fructose 6-phosphate phosphotransferase in carbohydrate metabolism of cold stored tubers of *Solanum tuberosum* cv. Bintje. *Plant Physiol.*, 95: 1243-1249.

DEGLI AGOSTI, R., E. VAN PRAAG & H. GREPPIN. 1992. Effect of chloride ions on the kinetic parameters of the potato tuber and mung bean pyrophosphate-dependent phosphofructokinase. *Biochem. Int.*, 26: 707-713.

FOWLER, D.B. 1992. *Winter wheat production manual*. University of Saskatchewan, Saskatoon, Canada. Also at the INTERNET web site: http://www.usask.ca/agriculture/cropsci/winter_wheat/

HUBER, S.C. 1986. Fructose 2,6-bisphosphate as a regulatory molecule in plants. *Annu. Rev. Plant Physiol.*, 37: 122-146.

LEE, J. 1996. Hardy oats stand up to a cold world. *Agricultural Res.*, 44(12): 8.

LYONS-JOHNSON, D. 1996. Cool nightlife bad for tomatoes. *Agricultural Res.*, 44(11): 15.

POLLOCK, C.J. & T. AP REES. 1975. Effect of cold on glucose metabolism by callus and tubers of *Solanum tuberosum*. *Phytochem.*, 14: 1903-1906.

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

VAN PRAAG, E. 1996. *Fructose 2,6-bisphosphate: its role in the regulation of carbohydrate metabolism in Citrus fruits*. Ph.D. dissertation. The Hebrew University, Jerusalem, Israel, pp. 1-168.

VAN PRAAG, E. 1997. Use of 3-D computer modelling and kinetic studies to analyse grapefruit pyrophosphate-dependent phosphofructokinase. *Int. J. of Biol. Macromol.*, in press.

VAN PRAAG, E., D. MONOD, H. GREPPIN & R. DEGLI AGOSTI. 1997. Response of the carbohydrate metabolism and fructose-2,6-bisphosphate to environmental changes. Effect of different light treatments. *Bot. Helv.*, 106: 103-112.

VAN SCHAFTINGEN, E., B. LEDERER, R. BARTRONS & H.G. HERS. 1982. A kinetic study of pyrophosphate: fructose 6-phosphate phosphotransferase from potato tubers. *Eur. J. Biochem.*, 129: 191-195.

WEEDEN, N.F. & B.B. BUCHANAN. 1983. Leaf cytosolic fructose-1,6-bisphosphatase. A potential target site in low temperature stress. *Plant Physiol.*, 72: 259-261.