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SYSTEMIC STRESS EFFECT ON THE SUGAR METABOLISM UNDER PHOTOPERIODIC CONSTRAINT

BY

Robert DEGLI AGOSTI* & Hubert GREPPIN

Abstract

Systemic stress effect on the sugar metabolism under photoperiodic constraint. - Spinach plants maintained in a short day photoperiod of 8:16 (L:D) remain vegetative. Transition to flowering (induction) may be initiated by extending the light phase of the photoperiod (transfer). We have examined the free sugar content during this transition and observed a sudden and considerable increase (in the order of 3 to 10 times the baseline) in the concentration of glucose already 3-5 h after the start of light extension. Various sources of stress affect this response. Pricks and cuts, as well as a brief, strong increase of an atmospheric pollutant such as ozone turned out to be inhibitors. At the time of the experiments (4 weeks), spinach plants possess two symmetrical and fully developed primary leaves. The pricking of one leaf just before the transfer treatment inhibits this particular carbohydrate response, not only in the directly wounded leaf but also in the adjacent unwounded leaf. The transmission of the stress effect from one leaf to the other is undeniable for both glucose and saccharose, it implies the existence of a systemic mechanism concerning photoperiodic-controlled carbohydrate metabolism in plants.

Key-words: glucose, photoperiodism, Spinacia olereacea, stress, sugars, systemic, wounding.

Abbreviations: ATP, Adenosine triphosphate; F.W., Fresh weight; NADPH, Nicotinamide adenine dinucleotide phosphate (reduced form); NADP⁺, Nicotinamide adenine dinucleotide phosphate (oxidised form); SD, Short day; T, Transfer; Tris, Tris (hydroxymethyl) –aminomethane.

INTRODUCTION

The interaction between plants and sunlight are essential for life on Earth. Indeed, thanks to the photosynthetic process, energy in the form of light is captured in order to be fixed in the form of organic molecules which constitute the basis of the construction of all living beings, as well as in their structure and as a source of energy. We usually symbolise this process by the equation:

 $\begin{array}{c} \text{Light (energy)} \\ 6 \text{ CO}_2 + 6 \text{ H}_2 \text{O} &\longrightarrow \text{C}_6 \text{H}_{12} \text{O}_6 \text{ (sugars)} + 6 \text{ O}_2 \end{array}$

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Within the linear working limits of such a system, the greater the amount of CO_2 , the greater will be the amount of sugar synthesised (quantitative aspect). However, the interactions with light are not only quantitative, but also qualitative. For example, specific pigments absorb particular wavelengths of the spectrum in order to engage adapted physiological processes (ex. Phytochrome). Another important example as far as the quality of the light is concerned is that of photoperiodism. This is the capacity of certain plants to measure the relative period of light received per day, which conditions their flowering. Simply speaking, one can distinguish short day plants, which flower only when the period of darkness goes beyond a certain limit, and those which require a longer light phase (long day plants). Spinach belongs to the latter category, and is the object of this article (VAN OORSCHOT, 1960; PARLEVLIET, 1966).

Given that the primary effect of light described above is that of photosynthesis which leads to the formation of sugars, we have for a long time been interested in studying the dynamics of sugars in the very first moments of a light phase extended until it is capable of inducing flowering in spinach. In this plant, the free sugars detected are essentially saccharose, glucose, and fructose (DEGLI AGOSTI & GREPPIN, 1987a). During these experiments, we showed that the extension of the photoperiod was linked to a sudden and considerable (of the order of 3 to 10 times) increase in the concentration in glucose and fructose in many parts of the plant once a critical period of light had been reached (DEGLI AGOSTI, 1985). This effect has also been observed in other plants (DEGLI AGOSTI *et al.*, 1989).

However, during the numerous manipulations, we also observed a certain number of experiments during which these increases did not occur. A closer examination of the conditions linked to these experiments lead us to demonstrate the inhibitory effect of stress on these responses as well as the property of transmission of this stress to nonaffected parts of the plant (systemic effect). In this article, we describe the experiments which prove these properties.

MATERIAL AND METHODS

Spinach (*Spinacia oleracea* L. cv. Nobel) is cultivated in a short day photoperiod (SD). The light was switched on at 08 h, and off at 16 h (local time), (8:16 L:D). Germination and growth take place in phytotrons (T: $20 \pm 0.5^{\circ}$ C; rH: 70 ± 5 % during light and 50 ± 5 % during darkness). The illumination given by the neon tubes (40 W Sylvania "Daylight" F40T12) is 6000 lux at the average level of the leaves, which is equivalent to an energy of 20.6 Wm⁻² (400-700 nm, ISCO spectroradiotometer). The transfer (T) takes place by extending the light phase of the short day from 16 h local time onwards, thus eliminating the dark phase.

The extraction of soluble sugars is carried out on 4 week old plants by introducing 1 cm cut segments of petioles or discs (3 mm diameter) of primary leaves in glass tubes containing 80 % (v/v) ethanol. The glass tubes are sealed and put in a warm bath (100°C) for 15-30 min. This extract is stable (for more than a month) and allows a total extraction of the sugars being examined.

Two different kinds of extraction were performed. The first was with 16 plants grouped for one extract. This corresponds to $2 \times 2 \times 16$ discs and to $1 \times 2 \times 16$ petioles segments. The second method consisted to obtain 4 extracts with 4 plants each ($2 \times 2 \times 4$ discs, $1 \times 2 \times 4$ segments). In the latter situation, results are expressed with their mathematical mean and standard deviation on the figures. In bilateral extractions the number of discs and segments are divided by a factor of two. For both methods, the amount of ethanol was 10 ml for about 1 g F.W.

The glucose is specifically measured by an enzymatic method (BERGMEYER *et al.*, 1974) using hexokinase (HK, Boehringer Mannheim) and glucose-6-phosphate dehydrogenase (G-6-PDH, Boehringer Manheim). Fluorescence due to the increase in the concentration of the NADPH cofactor is measured using a spectrofluorometer (Aminco-Bowmann spectrofluorometer equipped with an Aminco photon-counter) connected to a recorder (Linear recorder, Linear Instr. Corp.). The excitation and the emission wavelengths are, respectively, 340 nm and 480 nm.

The reaction medium for the fluorescence measurements is the following: the extract which is to be dosed is added to the buffer (Tris-HCl 0.05 M, MgCl₂ 2 mM at pH 8.1, with ATP 0.3 mM, NADP⁺ 0.05 mM) containing 0.7 U/ml of G-6-PDH. Endogenous fluorescence is noted (zero level) and subtracted from the fluorescence obtained 3-5 min after adding HK (final concentration of 3.5 U/ml). The fluorescence is converted into μ moles of glucose by refering to a standard scale and expressed per unit of fresh weight (g F.W.).

The saccharose is measured by the dosage of glucose after hydrolysis with invertase in an acid medium. Interference caused by the glucose present in the extract is eliminated by destroying the reducing hexoses in a strongly alkaline medium where saccharose is stable (VAN HANDEL, 1968). The alcoholic extract is dry evaporated (95°C), NaOH 0.1 M is added and then incubated at 95°C for 5 min in sealed tubes. The invertase (β -fructosidase, Boehringer, Manheim) at a final concentration of 50 U/ml dissolved in a Na-acetate buffer (0.1 M, pH 4.5) is added. After 20 min at room temperature, the hydrolysis of the saccharose is complete. The pH is then readjusted to 8.1 with a Tris 2 M solution. In all the cases measured, the production of glucose is equimolar with that of fructose. The addition of internal standards show that there is no degradation of these sugars during these procedures (for more details, see DEGLI AGOSTI & GREPPIN, 1987a).

RESULTS AND DISCUSSION

Fig. 1 shows the evolution of glucose in 4 week old spinach plants when they are transferred from SD to continuous light. In "optimal" conditions (no stress, Fig. 1, curve A), we observe the kinetics of the increase of glucose concentration during the 8 hour SD light phase, which leads to a saturation point. In SD, at 16 h, the light is switched off and the glucose concentration decreases gradually (results not shown here, see DEGLI AGOSTI, 1985; DEGLI AGOSTI *et al.*, 1990). On the other hand, when plants were put



Fig. 1.

Time course of the glucose content in 4 week old spinach petioles during the light phase of a short day (SD) photoperiod and during the transfer from SD to continuous light (T). A: in normal non-stressed conditions. B: with plants stressed during the SD light period (see text).

under stress (in this case, they were moved from one phytotron cell to another during the SD light phase immediately preceding the transfer treatment in a cold winter season), we observed that this increase did not occur (Fig. 1, curve B). In this case, the glucose stays at practically the same level as that of 16 h. Before understanding the effect of different types of stress, numerous (61) experiments had already been carried out over a period of two years. In 44 cases (72 %) we observed type A curves (Fig. 1) and in 17 cases, curves similar to case B (Fig. 1). Afterwards, particular precautions were taken to minimise any evident source of stress. These precautions turned out to be essential to reduce type B responses, as it will be shown later (Fig. 2). Other possible sources of stress could also be considered, such as the effect of low or high temperatures or the presence of parasitic or pathogenic agents.

In order to specify this stress effect, we carried out the following experiment: different proportions of leaf blade were cut at 16 h in the case where the plants had been prestressed (at 09 h in the morning) with 4 pricks on both primary leafs or in the case where the plants had not been stressed at all before. The results (Fig. 3 A & B) show that in the case of the non-stressed plants, cutting less than a quarter of the leaf blade leads to a decrease in total petiolar glucose instead of the usual increase. Furthermore, in prestressed plants we observe an interesting linear dependence between the petiolar



Treatments at 16 h



FIG. 2.

Effects of different "stressful" treatments effected at 16 h (local time) on the glucose increase in transfer conditions (T) at 23 h in 4 week old spinach petioles. The glucose content is expressed as percentages relative to untreated plants. In the latter, glucose concentrations were 1.5 μ mole / g F.W. at 16 h and of 5.2 μ mole / g F.W. at 23 h in transfer (T) conditions.

glucose content and the surface area of the leaf (Fig. 3 B). This result strongly suggests that there is a different management of at least two different pools of glucose in the spinach petiole. In addition to other experiments concerning compartmental analyses of these pools of glucose (DEGLI AGOSTI & GREPPIN, 1989), as well as through a mathematical modelling and dynamic simulation of glucose in the petiole (DEGLI AGOSTI & GREPPIN, 1987b), these results support the hypothesis of a specific control of a particular pool of glucose. More precisely, the glucose pool that is stress sensitive and which is in some yet unknown way linked to a biological clock (DEGLI AGOSTI & GREPPIN, 1988) and photoperiodism. All these observations suggest that the increase in the concentration of glucose has its origin in the control of the compartmentation of saccharose in the vacuoles of the cells (DEGLI AGOSTI, 1985; DEGLI AGOSTI & GREPPIN, 1989).

If one examines in detail the results shown in Fig. 2, one can observe that cutting the cotyledons affects the response measured on the leaf petioles. this seems to point to the existence of a mechanism of transmission from one point to the next in the plant: the wound site (cotyledons) and the site of measurement (petiole, leaf) are distant one from the other. At an age of 4 weeks, the spinach plant has two entirely developed and symmetrical primary leaves. We therefore undertook to "stress" only one of the two leaves and to examine whether or not the adjacent intact leaf was affected by these treatments. Fig. 4 shows that pricks (1 - 4) on the leaf lead to a modification of the



FIG. 3.

Effect of cutting different proportions of the leaf blade at 16 h on the subsequent glucose content increase during transfer to continuous light (T) in 4 week old spinach petioles. The glucose content is expressed as a percentage of that of untreated plants (100% of remaining leaf area). A: Plants were in normal conditions. B: Plants were previously stressed during the light phase of the SD at 09 h (local time) by 4 pricks on both primary leaves. In the latter condition there was no significant glucose increase between 16 h and 23 h (T23h), whereas for unstressed plants there is a significant increase between the glucose content at 16h (SD16h) and 23 h (T23h).

response to the photoperiodic transfer in the adjacent petiole. We then studied in greater detail if this effect was perceptible not only in the glucose, but also in the saccharose concentrations and also if it was also present at the level of the leaves. Fig. 5 shows that a processing of the data expressed as a percentage of the increase of the unstressed control (Fig. 6), illustrates this result in even clearer terms. In all cases, the response of the leaves and the petioles of the pricked side to an elongation of the photoperiod (the sharp increase in sugars) is almost completely suppressed. A transmission was present, since on the non-pricked sides, this response was clearly and considerably diminished.

Important pathways for the circulation of matter (chemicals) in plants include the phloem for sugars, amino acids and some hormones, and the xylem for water and inorganic nutrients (e.g. MOORBY, 1981). The flux in the xylem goes mainly from the roots to the leaves where water leaves the plant by transpiration. In the phloem, fluxes are possible in either direction depending on the source-sink situation of the tissue. A young leaf is a sink, because it needs organic matter to sustain its development, which can not be fulfilled by photosynthesis alone. Once the surface of the leaf is greater than 50% of its final size, it becomes a net exporter (see references cited on p. 411 in DEGLI AGOSTI *et al.*, 1990). In our situation, in spinach, the leaves have 100% of their final





Transmission of the inhibitory stress effect on the glucose content increase in 4 week old spinach petioles. Glucose concentration is expressed as a percentage of glucose level in unstressed plants. SD glucose level in spinach petioles at 16 h (SD16h, \blacksquare) is shown as control. At 16 h leaves were pricked on only one of the two symmetrical primary leaves. Extraction was done at 23 h and the petioles of the treated leaf (\bigcirc) and adjacent untreated leaves (\bigcirc) were extracted separately. Zero pricks correspond to the control treatment.



FIG. 5.

Glucose and saccharose concentrations in petioles and leaves of 4 week old spinach plants at 16 h in SD (SD16h) and at 23 h after transfer to continuous light (T23h). Control plants are shown in the middle of the figure. Stressed plants (4 pricks in only one of the two symmetrical spinach leaf blades at 16 h) are shown on either side. On the left, concentrations in the pricked part of the plants, whereas on the right side of the figure, the concentrations are those of the unstressed part of the plant.



FIG. 6.

Same as Fig. 5, however, the concentrations are expressed as percentages of the control plants sugars content increase between SD 16 h and T 23 h in spinach petioles and leaves. A transmission of the inhibitory stress effect is clearly visible.

size (FROSCH et al., 1986), they export organic matter to roots, apex, young leaves, stem and petioles. In our situation, it is therefore unlikely that transmission would be effected via the phloem or xylem. Indeed, 4 week old spinach leaves are exporting metabolites. Thus one could easily explain how a signal could be exported from the wounded leaf but surely not how the unwounded leaf could import it. The same reasoning holds more or less true in the reverse way for the xylem transport. The existence of rapid interorgan information transmission in plants has been the object of early works which have shown that different stimuli (light, pricking / wounding) can have interorgan effects, which can not be always explained by transport via phloem or xylem (DESBIEZ, 1971; PENEL & GREPPIN, 1975; OELZE-KAROW & MOHR, 1974; DE GREEF & CAUBERGS, 1972; ERNER et al., 1980). More recently, it has been shown that various genes can be triggered in a distant unwounded part of a plant (e.g. GATZ, 1997). Sometimes the nature of the transmission seems to involve chemicals (e.g. abscisic acid, jasmonic acid) or / and bioelectrical propagating signals (e.g. GATZ, 1997; HERDE et al., 1998; DAVIES, 1987). We would like to suggest an intercellular bioelectrical route of transmission as a working hypothesis, but clearly much remains to be done to answer to this question.

In plants, sugars are not only the products of photosynthesis and the supply of both the energy and buildings blocks for heterotrophic or sink tissues, but they also act as gene activity modulators / regulators (KOCH, 1996; SMEEKENS & ROOK, 1997; SMEEKENS, 1998; JANG & SHEEN, 1997, JANG *et al.*, 1997). In addition to this, it also known that sugars can enhance or even trigger the division of apical meristem cells which precedes floral meristem differentiation (KOCH, 1996; BERNIER *et al.*, 1993). We have been able to demonstrate an increase in glucose in spinach (and other plants) concomitant to photoperiodic floral induction for a long time now (DEGLI AGOSTI, 1985); we can therefore realistically speculate that glucose could be one of the very first molecules, which is able to trigger the flowering process in plants.

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RÉSUMÉ

EFFET SYSTÉMIQUE DU STRESS SUR LE MÉTABOLISME DES SUCRES SOUS CONTRAINTE PHOTOPÉRIODIQUE

Les plantes d'épinard maintenues dans une photopériode de jour court (8:16 L:D) restent à l'état végétatif. La transition à l'état floral (induction) peut être déclenchée par un allongement de la durée de la phase lumineuse (transfert). Nous avons examiné les teneurs en sucres libres lors de cette transition. Nous avons observé une augmentation brusque et importante (de l'ordre de 3 à 10 fois le niveau de base) dans la concentration en glucose déjà après 3-5h après le début de l'allongement de la phase lumineuse. Des stress de natures diverses modifient cette réponse. Des piqûres, des coupures, mais également une augmentation brève en un polluant atmosphérique comme l'ozone se sont révélés inhibiteurs. Au moment de l'expérience (4 semaines), l'épinard possède 2 feuilles primaires entièrement développées et symétriques. Lorsqu'on pique uniquement l'une de ces deux feuilles juste avant le transfert, cette réponse particulière des sucres est inhibée non seulement au niveau de la feuille stressée, mais également dans la feuille adjacente non blessée. La transmission de l'effet de stress d'une feuille à l'autre est indéniable aussi bien pour le saccharose que pour le glucose, elle implique l'existence d'un mécanisme systémique concernant la réponse du métabolisme des sucres sous contrainte photopériodique.

Mots-clefs: glucose, photopériodisme, *Spinacia olereacea*, stress, sucres, systémique.

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