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Photoperiodic floral induction and glucose content changes in spinach, mustard and *Chenopodium rubrum* plants

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Abstract

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In long day plants such as spinach or mustard cultivated under short-day conditions (8 h of light), the lengthening of the photoperiod results in a very large increase in glucose content which starts when the approximate floral photoperiod is reached. In the short day plant *Chenopodium rubrum* subjected to a transfer from continuous light to darkness, the glucose content decreases according to a mirror image of what is observed in the long day plants. These results suggest that photoperiodic flower induction is characterized by a common modification of the carbohydrate metabolism and/or by a change in their compartmentation.

Introduction

In mustard (*Sinapis alba* L.), a long day plant (LDP), photoperiodic induction of the flowering process was shown to be accompanied by an increase in total free sugar concentration (Bodson 1977, Bodson & Bernier 1985). Similarly, the glucose and fructose content in the petioles of 4-week old spinach plants (LDP) was shown to increase during the light phase and to decrease during the night of a short day (SD) of 8 h light. Moreover, the lengthening of the photoperiod resulted, after 8 h supplementary light, in a dramatic increase in the glucose and fructose level (580% and 900%, respectively; see Degli Agosti & Greppin 1987a). However, the quantitative and qualitative role of these sugars during the very early phase (hours) of flower induction was not studied. It was thus considered to be of interest to better characterize the time-dependent changes in the glucose content in spinach plants and to compare these patterns with other plants having specific day or night length requirements for their floral induction.

Material and methods

Spinacia oleracea L. cv. Nobel (spinach), a long day plant (Parlevliet 1966), was cultivated on soil, four plants to a pot, in growth chambers under standard irradiation

conditions (20.6 W m^{-2}) provided by Sylvania fluorescent lamps "daylight" F40T12, 40 W), temperature (20 ± 0.5 °C) and humidity (70 ± 5% relative humidity during the day and 50 ± 5% during the night). The vegetative plants were used after 4 weeks of growth in short days (SD) of 8 h light and 16 h darkness. Seedlings or adult vegetative plants of *Sinapis alba* L. (mustard), also a LDP (Bernier et al. 1981), were respectively used after either one week or two months of growth in the same conditions as for spinach plants. Seeds of *Chenopodium rubrum* L. ecotype 184, a short day plant (SDP), were planted on an agarized Hoagland's medium (see Cumming 1969) in transparent plastic boxes. The seedlings were obtained by subjecting the seeds to cycles of 12 h light at 32.5 °C and 12 h darkness at 10 °C for 4½ days followed by 24 h at 20 °C in continuous light (LL).

Floral induction was obtained by transferring the LDPs from SD to LL and the SDP from LL to darkness. Only the light regimes were modified since removing the plants from one growth chamber to another resulted in a diminished or delayed response. An additional photoperiodic treatment, the displaced short day (DSD), was tested with the mustard seedlings. The SD night period was lengthened up to 24 h total darkness and the experiment started by giving the light period from 16 to 24 hrs local time.

Glucose was extracted from known amounts (fresh weight, FW) of petioles or cotyledons. The tissues were dipped in glass tubes containing about 10 ml of 80% ethanol in water per g FW. The tubes were tightly sealed and heated to 100 °C for 15–30 min in a water bath. Preliminary experiments have shown that this method allowed a complete extraction of glucose. Glucose was assayed with the specific enzymatic assay described by Bergmeyer et al. (1974). The method was adapted for measurement with a spectrofluorimeter (Aminco Bowman, American Instrument Co, Silver Spring, USA) in order to increase its sensitivity (Degli Agosti and Greppin 1987a). The extract was added to the assay buffer (0.05 M Tris-HCl, pH 8.1, 2 mM $MgCl_2$, 3 mM ATP, 0.05 mM NADP and 0.7 U/ml glucose-6-phosphate dehydrogenase), and the endogenous fluorescence measured (zeroing). The reaction was then started by adding 3.5 U/ml (final concentration) of hexokinase. The NADPH fluorescence was measured during 3–5 min and converted to µmoles of glucose by using a calibration curve established with pure glucose. Tris buffer and $MgCl_2$ were from Merck (Darmstadt, FRG) and all other chemicals from Boehringer (Mannheim, FRG).

Each experiment was repeated at least 3 times with different batches of plants. The patterns of the time course in glucose content were very stable from experiment to experiment. Data shown are those from one representative set of experiments where the coefficients of variation were about 15% for each time-point.

Results

In spinach plants cultivated in SDs (fig. 1), the glucose content of the petioles and limbs increased significantly during the light period, reaching a steady state value after 2 to 4 h light, and decreased rapidly during the night period. The transfer from SD to LL was marked, after 8 to 10 h total light, by a rapid and very large glucose increase in both limbs and petioles. The effect of a DSD treatment (fig. 1 B) resulted in a very similar pattern as compared to the transfer treatment but without any lengthening of the photoperiod.

The same kind of pattern was obtained with one-week old mustard seedlings (fig. 2). In the petioles of adult (two months) mustard plants, the glucose content only presented slight variations during the SD but started to increase only 3 to 4 h after transfer from

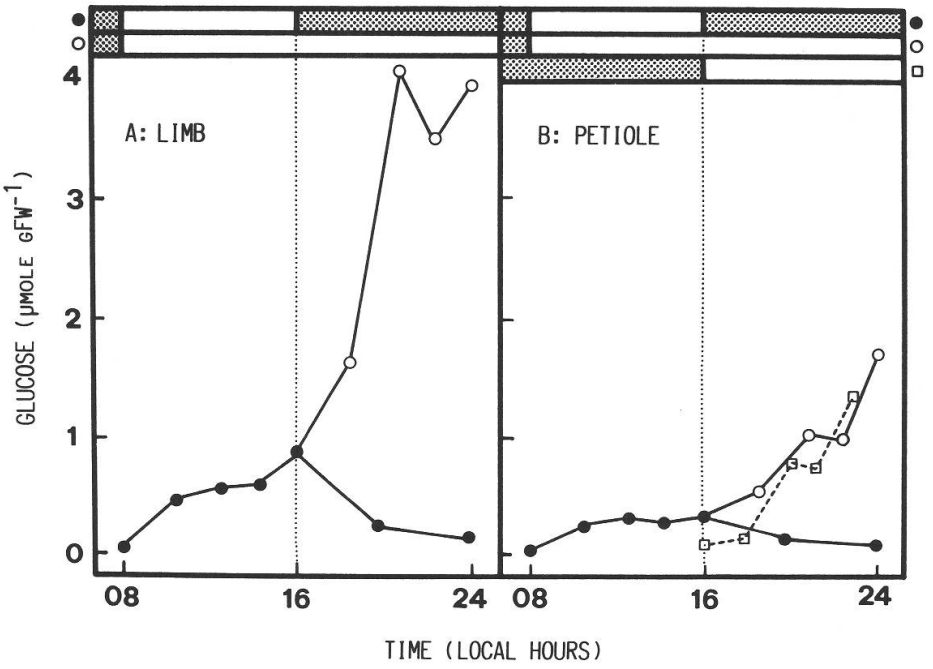

Fig. 1. Time course for the glucose content of the limbs (A) and the petioles (B) of 4 week-old spinach plants during the short day (●), and the transfer to continuous illumination (○) and the displaced short day (DSD, □).

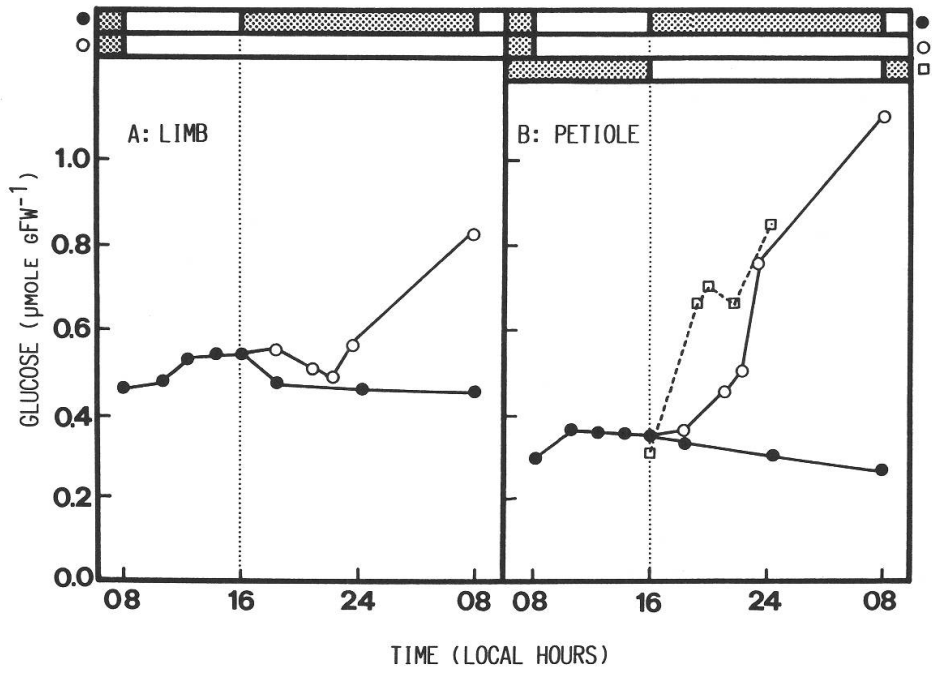

Fig. 2. Time course for the glucose content of (A) the cotyledonary limbs and (B) the petioles of one-week old mustard seedlings during the short day (●), the transfer to continuous illumination (○) and the displaced short day (DSD, □).

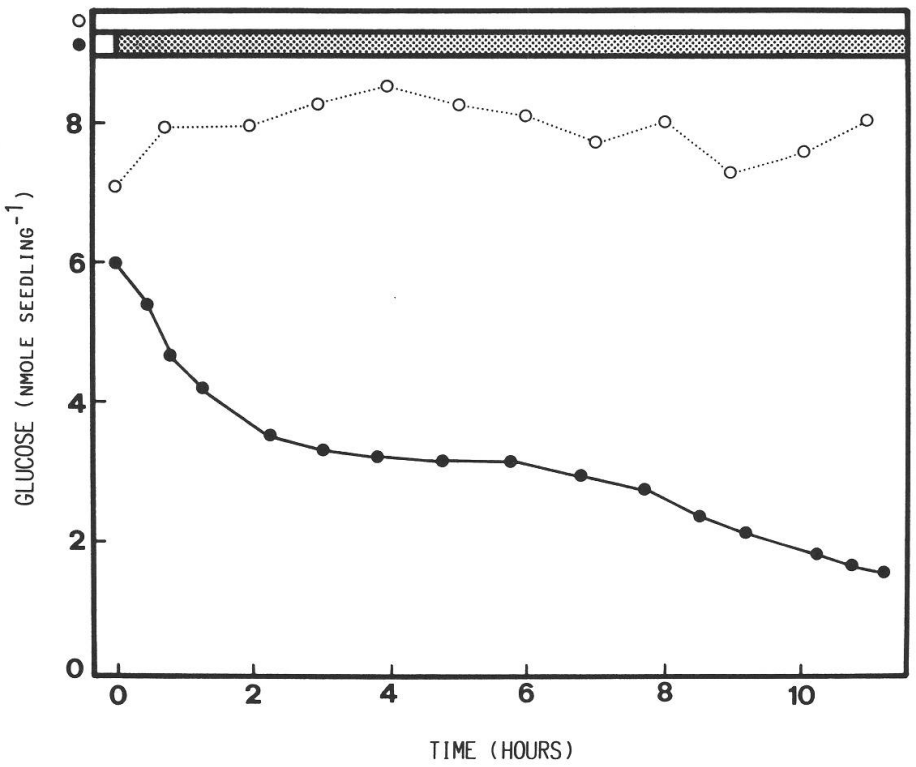

Fig. 3. Time course for the glucose content of 5½ day-old cotyledons of *Chenopodium rubrum* seedlings in continuous light (o) and during the transfer to darkness (●). Moving mean over three points.

SD to LL (results not shown, Degli Agosti 1985). The effect of a DSD (fig. 2 B) was also marked by a sharp and rapid increase in the glucose content which was triggered by the displaced light-on signal.

For the SDP *Chenopodium rubrum* cultivated in LL (fig. 3), the glucose content of the cotyledons was oscillating around a mean value, whereas the transfer to darkness was marked by its decrease according to a "sigmoidal" curve having its inflexion point after about 5 h darkness. This kind of pattern is very similar to the respiration measurements made in *Chenopodium polyspermum* by Mousseau & Louason (1976) and Mousseau (1977) in the same photoperiodic conditions. As shown in fig. 4, this response can be considered as a mirror image of that observed for the LDPs.

Discussion

When LDPs such as spinach or mustard were subjected to a lengthening of the SD light period, a very large increase in the glucose content of the limbs and petioles (figs. 1 and 2) started a few hours after the extension of the short-day's light phase. Preliminary results (Degli Agosti 1985) have shown that this kind of response is also present in *Coleus blumei* Benth. cv. golden bedder, another LDP (Dupperex 1965). Reconsideration of the free sugar measurements made by Bodson (1977) showed, despite the few time-points, that such a pattern was present in mustard leaves subjected to flower induction but with

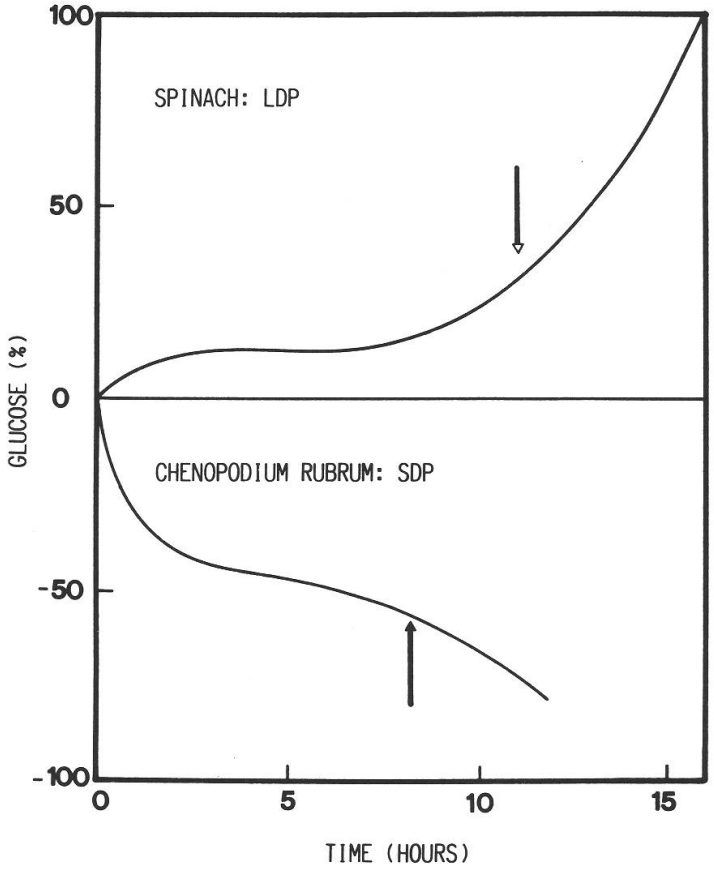

Fig. 4. Comparison of the glucose content of the petioles of spinach plants during the transfer from short days to continuous illumination (calculated in % of the amount after 16 h total light) with that of *Chenopodium rubrum* seedlings transferred from continuous light to darkness (in % of the amount since the light off signal). Time is given in hours from the moment where the LDP received light and SDP darkness. Arrows indicate approximate critical photo-(▽) and nycto-(▲) period.

a very low amplitude which can be attributed to the unspecific assay method employed. A similar behaviour was also deduced from the results of Kerr et al. (1985) with soja leaves where an increase in different hexoses, including glucose, began 8 h after the lengthening of the photoperiod.

In spinach, the metabolism of sucrose could be implied as a metabolic source for this glucose increase. Degli Agosti (1985) showed that this effect in petioles was not due to starch hydrolysis but to the photosynthetic activity in the leaf blades. The time course for the changes in fructose and sucrose content of the petioles during floral induction clearly indicated that at least part of the sucrose pool was the source for the glucose and fructose increase. In the leaf blade sucrose can be temporarily stored in the vacuoles (Fisher & Outlaw 1979, Gerhardt & Heldt 1984, Foyer 1987) where it could be hydrolyzed (Avigad 1982). Results from a mathematical analysis (Degli Agosti and Greppin 1987b) and from an in vitro analysis of glucose release from spinach petioles (Degli Agosti and Greppin 1989) also suggest a vacuolar localisation of the phenomenon in the petioles. These data would imply a photoperiodically controlled delocalisation of sucrose out of the phloem to parenchymatic cells. At a molecular level we propose a change in membrane properties, especially in the sucrose-translocator activities and perhaps also in plasmodesmata, since they play a role in phloem unloading (Biao Ding et al. 1988).

In this context it is worthwhile to mention that changes in membrane composition and properties have been detected during early induction phase of flowering in spinach (Auderset et al. 1986, Penel et al. 1988).

In the case of the SDP *Chenopodium rubrum,* although there was some similarity with the LDPs (fig. 4), the decrease in the glucose content of the cotyledons during the transfer from LL to darkness (fig. 3) could not be directly related to sucrose metabolism since starch hydrolysis was not ruled out as a possible metabolic source for it. It was nevertheless evident, as for the LDPs, that the metabolism of the free sugars and possibly their distribution in the different cell compartments (control of emptying in SDP and filling-up in LDP) and/or plants organs, was profoundly modified around the critical floral photoperiod (see fig. 4). In addition, the DSD effect on mustard and spinach plants (fig. 1 B and 2 B) clearly showed that the increase in glucose level upon transfer to LL was not attributable to a cumulative effect linked to the total duration of light, but to a qualitative phenomenon triggered at a certain phase of the SD period. It might thus be suggested that the modification of the glucose level is related to the coincidence between the presence of light (external factor) and an internal pre-determined specific organisation of carbohydrate metabolism phased by the preceding SDs periods. In spinach, the endogenous rhythmic movement of the leaves could be shown to coincide with the increase in glucose content of the petioles (Degli Agosti 1985, Degli Agosti and Greppin 1988), indicating the participation of a biological rhythm in this phenomenon. For spinach plants subjected to a transfer from SD to LL, the results can be related to the increase in energy and redox charge (Bonzon et al. 1981, 1983) in the leaves and to that in glucose-6-phosphate dehydrogenase activity in the apices (Gahan et al. 1979, Auderset et al. 1980). A similar comparison can be done with the increase in invertase activity and sucrose content in mustard apices during transition to flowering (Pryke and Bernier 1978, Bodson and Outlaw 1985). It was suggested that some sugars were part of the floral stimulus which is produced in the leaf blades and transported to the apices via the petioles (see Bernier et al. 1981). This hypothesis was already much debated by Bodson and Bernier (1985). The results presented in this study also suggest that the total glucose content of the petioles cannot simply be considered as a determinant factor for flower induction since it increased in the LDPs and decreased in the SDP. Any unifying hypothesis about the role of the free sugars in floral induction would most likely include a common modification of their metabolism and, possibly, a change in their cell and/or organ compartmentation.

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