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# Glucose-6-phosphate dehydrogenase activity in the shoot apices of *Spinacia oleracea* after the floral evocation in continuous light

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## RÉSUMÉ

ELTHOUKI, M., G. AUDERSET & H. GREPPIN (1985). Activité de la glucose-6-phosphate-deshydrogénase dans l'apex caulinaire de *Spinacia oleracea* après l'évocation florale en lumière continue. *Saussurea* 16: 1-5. En anglais, résumé français.

Une étude cytochimique quantitative de l'activité de la glucose-6-phosphate-deshydrogénase, marqueur de la voie des pentoses (PPP) a été faite sur des apex caulinaires de *Spinacia oleracea* âgés de 4 semaines et placés, soit en jours courts (8 h de lumière blanche et 16 h d'obscurité), soit transférés en lumière continue pour une période totale de 24 h, 48 h et 96 h. Il en résulte un doublement du taux de l'activité qui se maintient durant la période d'illumination au-delà de 24 h. Cette activité n'est pas modifiée de manière significative lorsque l'illumination est prolongée à 48 h; il apparaît une légère diminution après 96 h d'illumination continue. Ceci nous amène à confirmer que la glucose-6-phosphate-deshydrogénase est bien un marqueur précoce de l'induction florale et de l'état floral.

## ABSTRACT

ELTHOUKI, M., G. AUDERSET & H. GREPPIN (1985). Glucose-6-phosphate dehydrogenase activity in the shoot apices of *Spinacia oleracea* after the floral evocation in continuous light. *Saussurea* 16: 1-5. In English, French abstract.

A quantitative cytochemical study of glucose-6-phosphate dehydrogenase activity as a marker of the pentose phosphate pathway (PPP) was made on 4 weeks old shoot apices of *Spinacia oleracea* kept either on short day (8 h fluorescent white light and 16 h darkness) or transferred to continuous illumination for total period of 24 h, 48 h and 96 h. A doubling of the rate of activity occurs and is maintained during the periode of illumination up to 24 h. The activity was not significantly affected when the illumination period was prolonged to 48 h; but it seems to be slightly decreasing after 96 h of continuous illumination. It leads to the confirmation therefore that the glucose-6-phosphate dehydrogenase is an early marker of floral induction and the floral stage.

## Introduction

It was previously shown (GAHAN & al., 1978) that using G-6-PD and 6-phosphogluconate dehydrogenase as marker enzymes, the activity of the pentose phosphate pathway (PPP) was elevated in shoot apices of *Spinacia oleracea* after transfer from short daylength (8 h illumination) to continuous daylength for a total period of 24 h. These studies were based on the reaction of glucose-6-phosphate dehydrogenase (G-6-PD) which was chosen as a marker enzyme to measure the flux through the PPP since this pathway is regulated at the G-6-PD step, a fine control operating through the ratio of NADPH<sup>+</sup>/NADP (TURNER & TURNER, 1980). Thus any measurement changes in G-6-PD activity as shown cytochemically should reflect changes in flux through the PPP (NEWSHOLME & al., 1980).

The aim of the present study is to show how early the PPP activity is elevated on transferred plants to continuous illumination and if this increased activity could be maintained for a period of continuous illumination up to 96 h.

## Materials and methods

Plants of *S. oleracea* var. Nobel were grown for 4 weeks at 21°C under a short day regime (8 h fluorescent white light and 16 h darkness) with a relative humidity of 50% and 70%, respectively, during the light and the dark periods. They were then transferred to continuous illumination for a total of 24 h, 48 h and 96 h. Plants kept on a short day regime were used as controls. Plants from each group were selected for maximum morphological uniformity prior to removal of the shoot apices which were frozen (GAHAN & al., 1979) and sectioned (GAHAN & al., 1967). The unfixed longitudinal frozen sections were reacted for G-6-PD activity as a marker of the flux through the PPP (AUDERSET & al., 1980). The incubation medium contained 4.6 mM glucose-6-phosphate, 3.2 mM NADP and 3.7 mM nitroblue tetrazolium in 50 mM glycylglycine buffer at pH 7.8 (BUTCHER & al., 1980).

Polyvinyl alcohol B0S/140 (22% w/v) was added to the medium as a colloid stabilizer to prevent movement of the soluble enzyme from the cells (GAHAN & al., 1979; ALTMAN, 1980). The reaction in cells of the whole apex were monitored for 20 min at 26°C with a MPV2 of Leitz where an addition of scanning stage and computer control allows scanning and integrating microdensitometry. The rate of reaction was calculated from the linear regression line derived from plotting absolute integrated optical density units (AIOD) per  $\mu\text{m}^2$  of reacted cytoplasm vs time of the incubation.

## Results and discussion

An analysis of the rate of G-6-PD in transferred apices to continuous illumination for 24 h showed an increase of the activity.

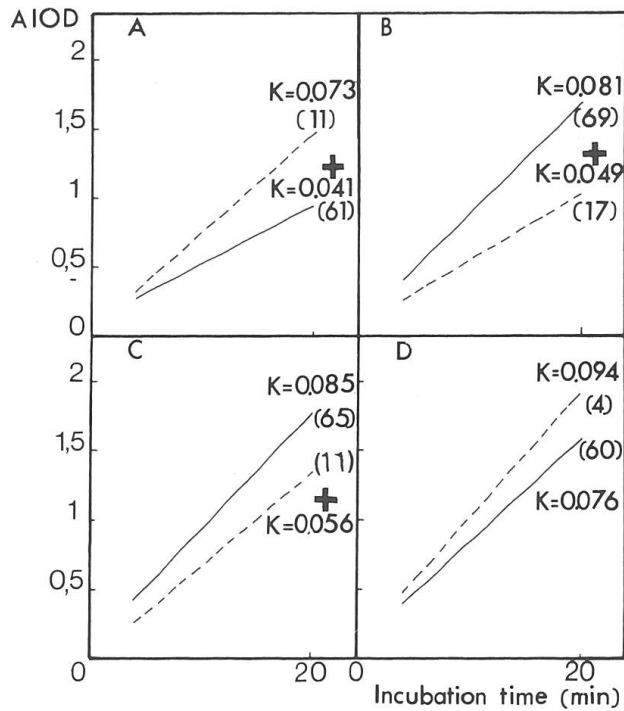
The mean rate of the activity of the plants transferred for 24 h (0.081) was approximately twice that of the control apices (0.042).

The rate of G-6-PD for the vegetative apices if always the same even 96 h after (0.042), as shown in table 1. Virtually, most of the apices showed increased activity.

Rates of activity of G-6-PD	SD	24 h C.L.	48 h C.L.	96 h C.L.
A°	0.042+ (61)*	0.081 (69)*	0.085 (65)*	0.077 (60)*
B°	0.073 (11)*	0.049+ (17)*	0.056+ (11)*	0.094 (4) *
CR	0.09939	0.09981	0.9916	0.09984

Rates are calculated from the slope (*m*) of the mean linear regression line ( $y = mx + c$ ) derived from measurements of each group of apices during a total incubation at 26°C for 20 min (measurement monitored each 2 min during incubation. \*Number in brackets indicate number of apices (this work was affected on 4 series of plants). °Apices in vegetative states.

Table 1. - The mean rates of G-6-PD in both vegetative and induced shoot apices of *S. oleracea* up to 96 h C.L.



———— the major portion of the apices;  
 - - - - - apices with an expected level below or above of the majority;  
 + vegetative apices.

$$K = \frac{\text{units of absolute integrated optical density}}{\mu\text{m}^2 \text{ reacted cytoplasm}}$$

Fig. 1. - G-6-PD activity graphs of absolute optical density/ $\mu\text{m}^2$  of reacted cytoplasm (AIOD) vs. time of incubation at 26°C.  
 A, vegetative apices (8 h light/16 h dark). B, apices transferred to continuous illumination for 24 h. C, apices transferred to continuous illumination for 48 h. D, apices transferred to continuous illumination for 96 h.

It is of interest to note that 11 apices out of 72 of vegetative apices have a level above that expected in the major portion of vegetative apices ( $A^\circ = 0.042^+$ ;  $B^\circ = 0.073$ ) [ $A^\circ$  = the major portion of the apices reacted for G-6-PD;  $B^\circ$  = apices have an expected level below or above the majority; + = vegetative apices]. A similar phenomenon also occurs in the apices induced by the transfer to continuous illumination. For the apices transferred for 24 h, 17 apices out of 86 have a rate of G-6-PD similar to those of vegetative apices ( $A^\circ = 0.069$ ;  $B^\circ = 0.049^+$ ); for the apices transferred for 48 h, 11 apices out of 76 have a level rate similar of those of vegetative apices ( $A^\circ = 0.085$ ;  $B^\circ = 0.056^+$ ). Only 4 apices in 64 of apices transferred to continuous illumination for 96 h have a level above that expected in other apices ( $A^\circ = 0.077$ ;  $B^\circ = 0.094$ ).

The observed difference in the case of floral induction depends upon the natural heterogeneity of the population of apices which in the case of *S. oleracea* is largely due to being dioecious (SUTO & SUGIYAMA, 1960; GREPPIN, 1965). These results show that *S. oleracea* is quantitative rather than qualitative long-day plant (SALISBURY, 1963; AUDERSET & al., 1980).

The implication is that by the time the apices from short day plants, become progressively induced, they have elevated G-6-PD levels and hence a greater flux through the PPP (TURNER & TURNER, 1980; NEWSHOLME & al., 1980); while this phenomenon occurs quickly in vegetative apices transferred to continuous illumination.

This observed elevated activity in apices after [24 h (0.081), 48 h (0.085)] of continuous illumination did not quite occur at the same level after 96 h of continuous illumination (0.077).

The high rate of G-6-PD in transferred apices is maintained as long as the central zone is not activated (mitosis). After the activation of this zone: 40-60 h after the transfer (AUDERSET & GREPPIN, 1976), the rate of the activity of G-6-PD decrease to about 10% of that after 24 h of transfer (0.082), but it is always approximately twice that of control apices (0.042).

So, it might be considered that the glucose-6-phosphate dehydrogenase is also a good marker of the floral stage as it is for the floral induction in the shoot apices of *Spinacia oleracea*.

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