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Cytological and histochemical study of glucose-6-phosphate dehydrogenase and the mitotic activities in the shoot apex of Spinacia oleracea var. Nobel during floral induction

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RÉSUMÉ

EL TOUKHI, M. G. AUDERSET & H. GREPPIN (1986). Etude cytologique et cytochimique des activités glucose-6-phosphate déshydrogénasique et mitotique dans l'apex caulinaire d'épinard (Spinacia oleracea var. Nobel) lors de l'induction florale. *Saussurea* 17: 93-102. En anglais, résumé français.

Une étude cytochimique quantitative de l'activité de la glucose-6-phosphate déshydrogénase, marqueur de l'activité de la voie des pentoses, et une étude de l'index mitotique ont été menées au niveau des différentes zones de l'apex caulinaire de *Spinacia oleracea,* var. Nobel, sous régime photopériodique de jours courts (plantes végétatives) et après transfert en jour continu pour des durées de 24, 40 et 48 heures. Après 24 heures de jour continu, l'activité G-6-PD de la zone centrale s'élève à un niveau deux fois plus élevé que celui enregistré chez les plantes de jours courts. Cette élévation est accompagnée d'une augmentation de l'index mitotique de cette même zone centro-apicale. Après 24 heures de transfert, toujours, ces deux activités restent inchangées au niveau de la zone latérale. Après 40 et 48 heures de lumière continue, l'activité G-6-PD et l'activité mitotique sont maintenues au niveau de la zone centrale alors qu'elles augmentent dans la zone latérale qui assure la préparation des bourgeons floraux.

ABSTRACT

EL TOUKHI, M., G. AUDERSET & H. GREPPIN (1986). Cytological and histochemical study of glucose-6-phosphate dehydrogenase and the mitotic activities in the shoot apex of Spinacia oleracea var. Nobel during floral induction. *Saussurea* 17: 93-102. In English, French abstract.

Both a quantitative cytochemical study of glucose-6-phosphate dehydrogenase (G-6-PD) activity as a marker of the pentose phosphate pathway (PPP) and the index of mitotic activity in the meristematic zones were carried out on the

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shoot apex of *S. oleracea*, kept either on short day (vegetative plants) or after transfer to 24 h, 40 h and 48 h long-day (continuous light). After 24 h long-day, measurement of G-6-PD activity showed that a high rate of this activity occurs (double of that of the short day) in the central zone accompanied by an increase of the mitotic activity, whereas these activities are still low in the lateral zone even after the transfer. After 40 h and 48 h long-day, both G-6-PD and the mitotic activities are maintainteed in the central zone, since they became most important in the lateral zone to assume the preparation of a floral bud initiation.

Introduction

The floral induction in the shoot apical meristem of *S. oleracea* can be achieved by subjecting 4-weeks old vegetative plants (short day plants) to a single 18 h long-day [1, 2]. The apical meristem of *S. oleracea* exhibits a typical cyto-histological zonation [1], a centrally located zone (the central zone) is surrounded by an approximately ring-shaped zone (the initiating zone or the lateral zone). During the vegetative growth of the plant, the leaves originate in organogenetic sites in the lateral zone. After floral induction, floral buds are produced by the new lateral zone of the floral apex which originate from the activated vegetative central zone. A third zone (the pith-rib meristem) lies below the central zone and induced ranks of flatted cells which function as a rib-meristem giving rise to the stem pith (Fig. 1).

It was previously shown [3] that using glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase as marker enzymes, the activity of the pentose phosphate path (PPP) was elevated in the shoot apices, after transfer

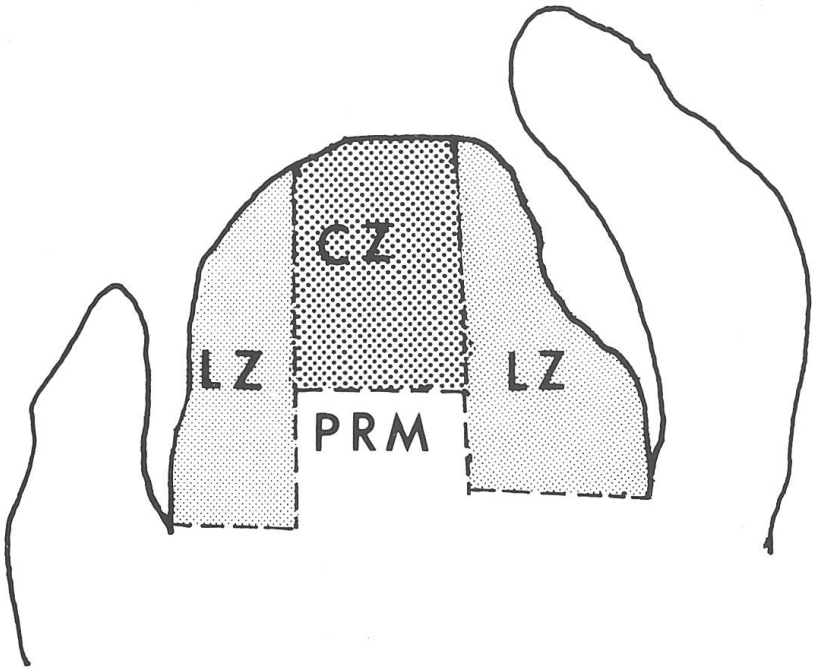

Fig. 1. – Longisection of a vegetative apical meristem of *S. oleracea*. The various meristematic zones are delineated (broken line).
LZ, lateral zone; **CZ**, central zone; **PRM**, Pith-rib meristem.

from short day (8 h illumination, 16 h dark) to continuous light for a total period of 24 h [4]. This activity was increased after 48 h and seemed to be maintained or slightly decreased after 96 h long-day [5].

The present paper concerns a cytological and cytochemical study of the shoot apices of *S. oleracea.* In particular the cellular distinction of G-6-PD activity levels compared with the zones of mitotic activity in the various regions of the shoot apex from vegetative and flowering induced plants.

It was thus found more interesting to consider separately both the enzymatic activity and the mitotic activity in each of the meristematic zones as it was done for the meristem as a whole [5].

The experiments described below were repeated three times.

Materials and methods

Plants of *S. oleracea* c.v. Nobel were grown for 4 weeks at 21°C and illuminated by means of fluorescent white light giving an intensity of 12,000 ergs $cm^{-2}s^{-1}$. The plants were maintained in the vegetative condition (8 h light, 16 h darkness) with a relative humidity of 50% and 70% respectively, during the light and dark periods. Some plants were then transferred to long day (continuous light) conditions for a total period of 24 h, 40 h and 48 h. Plants continuously kept in short day-regime were used as control. Plants from each group and of similar age were selected for maximum morphological uniformity prior to removal of the shoot apices, which were sampled at the end of each photoperiod treatment.

Histological method

The apices collected were fixed in formol-alcohol-acetic acid (FAA, v/v: 5/90/5) for at least 12 h, then dehydrated through an alcohol series and embedded in wax. Serial longitudinal sections, 5 μm thick were stained with Heidenhain's hematoxylin. The study was carried out on the median longi-section of each shoot apex. A camera drowing attached to a Wild M20 microscope at standard magnification was used to draw every section of each apex examined. Only the central and the lateral zones were considered. The different mitotic phases were recognized, counted and scored in a table in percent of the cells number of each zone.

Enzymatic reaction

Collected apices were frozen [3] and sectioned [6]. The unfixed frozen section was reacted for G-6-PD activity as a marker of the flux through the PPP [4]. The incubation medium contained 4.5 mM glucose-6-phosphate, 3.2 mM NAPD and 3.7 mM nitroblue tetrazolium in 50 mM glycylglycine buffer at pH 7.8 [7]. Polyvinyl alcohol BOS/140 (22% w/v) was added to the medium as a colloid stabilizer to prevent movement of the soluble enzyme from the cell [3, 8].

In order to evaluate the endogenous activity of G-6-PD, glucose-6-phosphate was omitted from the incubation medium. The section reacted for G-6-PD endogenous activity was removed from the cryostat and incubation medium was immediately added.

As the control, sections were reacted in a full test solution lacking NADP, or both glucose-6-phosphate and NADP.

Treatment of plant	*Lateral zone*	*Central zone*	*T value*		*F value*
Short day	9.12 ± 0.99%	11.0 ± 0.75%	4.254	a	1.718
24h long-day length	10.50 ± 1.19%	17.50 ± .75%	2.504	b	0.687
40h long-day length	31.75 ± 1.91%	18.25 ± 1.67%	1.111	c	0.307
48h long-day length	40.62 ± 2.92%	19.75 ± 1.48%	1.897	d	1.258

L.Z.: lateral zone
C.Z.: central zone
a: compared the mean of mitotic index value in L.Z and C.Z of short day apices
b: compared the mean of mitotic index value in L.Z of short day and L.Z of 24 h long-day apices
c: compared the mean of mitotic index value in C.Z of 24 h long-day and 40 h long-day apices
d: compared the mean of mitotic index value in C.Z of 40 h and 48 h long-day apices
$n = 7$

Table 1. – The index of the mitotic activity in both lateral and central zones of vegetative and induced shoot apices of *S. oleracea.*

	Rate of activity of G-6-PD			
	Vegetative	*24h L.D*	*40h L.D*	*48h L.D*
Lateral zone	0.0350 0.012*	0.0415 0.0086*	0.0695 0.0038*	0.0748 0.0028*
	(38)	(36)	(36)	(36)
CC	0.944	0.960	0.977	0.994
Central zone	0.0413 0.0068*	0.0785 0.0161*	0.0565 0.0020*	0.0482 0.018*
	(38)	(36)	(36)	(36)
CC	0.966	0.945	0.985	0.985

L.D: long-day length
CC: Pearson's correlation coefficient
Numbers in brackets indicate the number of apices
Rates are calculated from the slope (m) of the mean regression line ($y = mx + c$) derived from the measurement of each group of apices during a total incubation time of 20 min at 26°C (measurement monitored each 2 min at 26°C (measurement monitored each 2 min during incubation)
* Standard deviation from the means slope.

Table 2. – The mean rate of G-6-PD activity in both lateral and central zones, of vegetative and induced shoot apices of *S. oleracea* up to 48 th.

	Rate of activity of G-6-PD			
	Vegetative	*24h L.D*	*40h L.D*	*48h L.D*
Lateral zone	0.00701 0.0031*	0.00924 0.0036*	0.0148 0.0025*	0.0164 0.0082*
	(24)	(26)	(26)	(26)
CC	0.944	0.943	0.991	0.997
Central zone	0.00771 0.0060*	0.0184 0.0022*	0.0125 0.0034*	0.00746 0.0228*
	(24)	(25)	(26)	(26)
CC	0.996	0.995	0.947	0.993

L.D: long-day length
CC: Pearson's correlation coefficient
Numbers in brackets indicate the number of apices
Rates are calculated from the slope (m) of the mean regression line ($y = mx + c$) derived from the measurement of each group of apices during a total incubation time of 20 min at 26°C (measurement monitored each 2 min at 26°C during incubation
* Standard deviation from the mean slope.

Table 3. – The mean rate of endogenous activity of G-6-PD in both lateral and central zones of vegetative and induced shoot apices of *S. oleracea* up to 48h.

The reaction in cells of each zone of the apex was monitored for 20 min at 26°C with a MPV2 Leitz integrating microdensitometer [5]. The rate of reaction was calculated as in [6, 9].

Statistical analysis

Histological results: this experiment was performed three times and 6-8 apices were used every time. T-test and F-test (student and analysis of variances tests) were also performed for these results and their values were reported in table 1.

Enzymatic results: rate of G-6-PD activity was calculated for each apex where the correlation coefficient was always > 0.998. Then the mean rate was calculated for all the apices of each treatment and standard deviation was reported in Tables 2 and 3.

Results

Data from a particular treatment from one repeat compared with data from the corresponding treatment in the other repeats were all found very similar. To avoid overloading the results with too much data, however, only one set of data for the remaining treatment is shown.

3.1. Mitotic activity

A slight difference in the mitotic index was found within the central and lateral zones of control apices (short day apices) (Fig. 2). From the comparison of 24 h long-day induced apices and control apices, there was a significant rise of the mitotic activity in the central zone of 24 h long-day induced apices, while that of the lateral zone was slightly increased (no significant difference, T and F values, Table 1).

By the time, the mitotic activity rose sharply in the lateral zone (see 40 h long-day) and this rise continued reaching its high level after 48 h long-day, while that recorded within the central zone remained unchangeable.

3.2. G-6-PD activity

G-6-PD activity was low in the central zone and much lower in the lateral zone of the vegetative apices. A doubling of the rate of the activity was observed in the central zone of the induced apices by 24 h long-day (Figs. 3, 4) since that of the lateral zone did not significantly change (table 2). This increase of G-6-PD activity in early induced apices was decreased after 48 h long-day, whereas in the lateral zone an opposite result was obtained and the G-6-PD activity became more evident (Table 2).

Similar results were also obtained from the essays to evaluate G-6-PD endogenous activity when the substrat (glucose-6-phosphate) was omitted. Table 3 (Figs. 5, 6) summarize the changes in the rate of this endogenous activity in the vegetative, 24 h, 40 h and 48 h long-day induced apices.

Discussion

The high rate of G-6-PD activity in the central zone of 24 h long-day induced apices that by the time the apices from short day plants and transferred to continuous illumination become induced. They have elevated G-6-PD activity

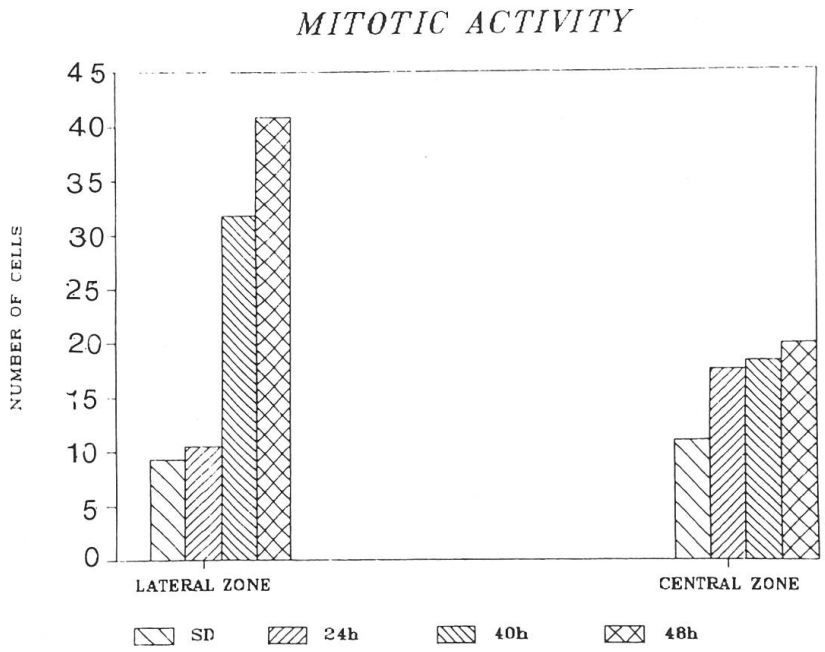

Fig. 2. – Mitotic index in the lateral and central zone of vegetative and induced apical meristem of *S. oleracea*.

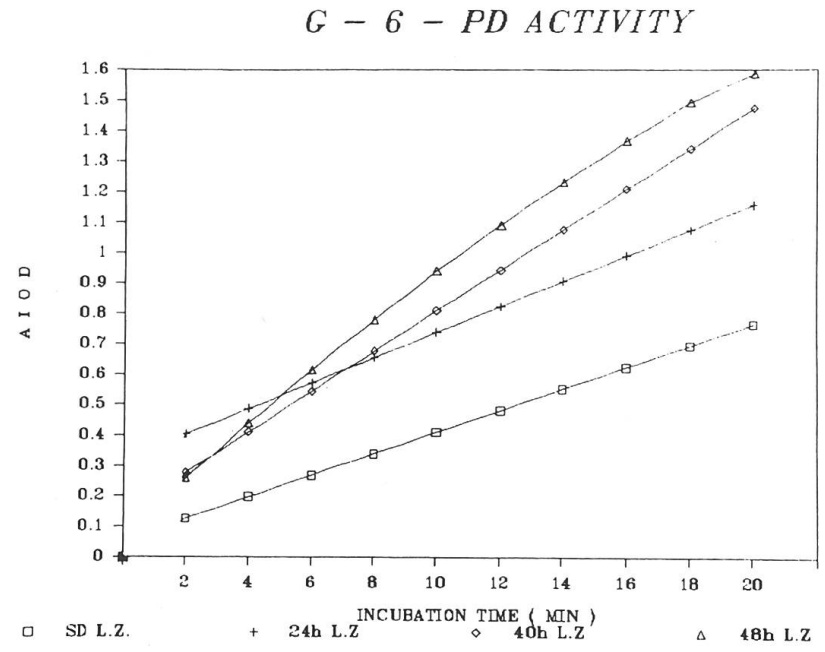

Fig. 3. – G-6-PD activity graph of absolute optical density/ μm^2 of reacted cytoplasm (AIOD) vs. time of incubation at 26°C in the lateral zone.
LZ, lateral zone; **SD,** short day apices; **24 h,** apices transferred to 24 h long-day length; **40 h,** apices transferred to 40 h long-day length; **48 h,** apices transferred to 48h long-day length.

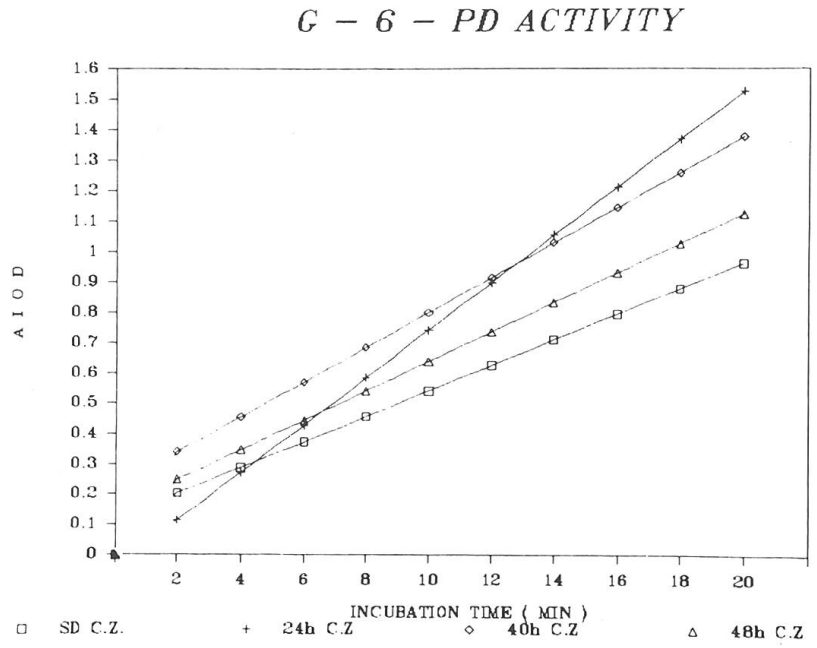

Fig. 4. – G-6-PD activity graph of absolute optical density/ μm^2 of reacted cytoplasm (AIOD) vs. time of incubation at 26°C in the central zone.
LZ, central zone; **SD,** short day apices; **24 h,** apices transferred to 24 h long-day length; **40 h,** apices transferred to 40 h long-day length; **48 h,** apices transferred to 48 h long-day length.

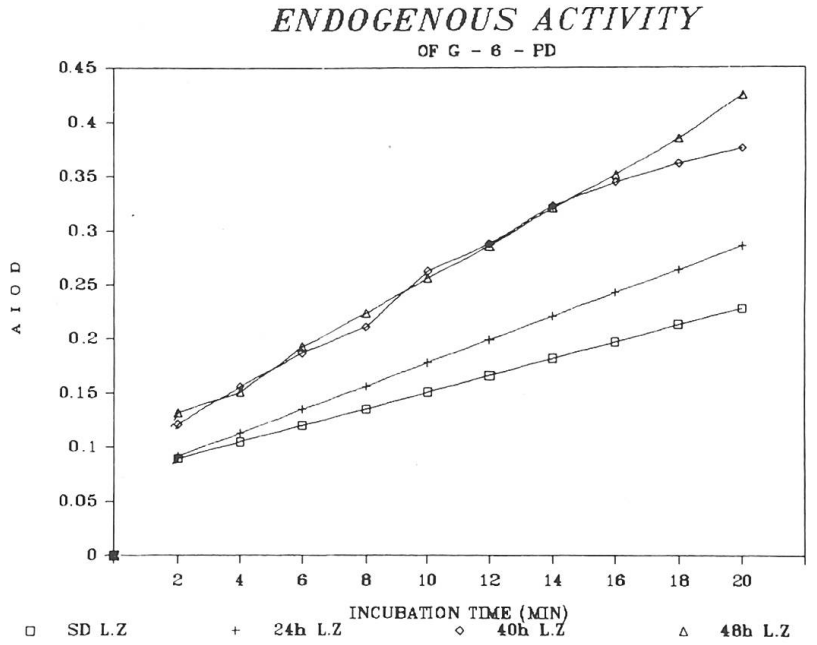

Fig. 5. – G-6-PD endogenous activity graph of absolute optical density/ μm^2 of reacted cytoplasm (AIOD) vs. time of incubation at 26°C in the lateral zone.
LZ, lateral zone; **SD,** short day apices; **24 h,** apices transferred to 24 h long-day length; **40 h,** apices transferred to 40 h long-day length; **48 h,** apices transferred to 48 h long-day length.

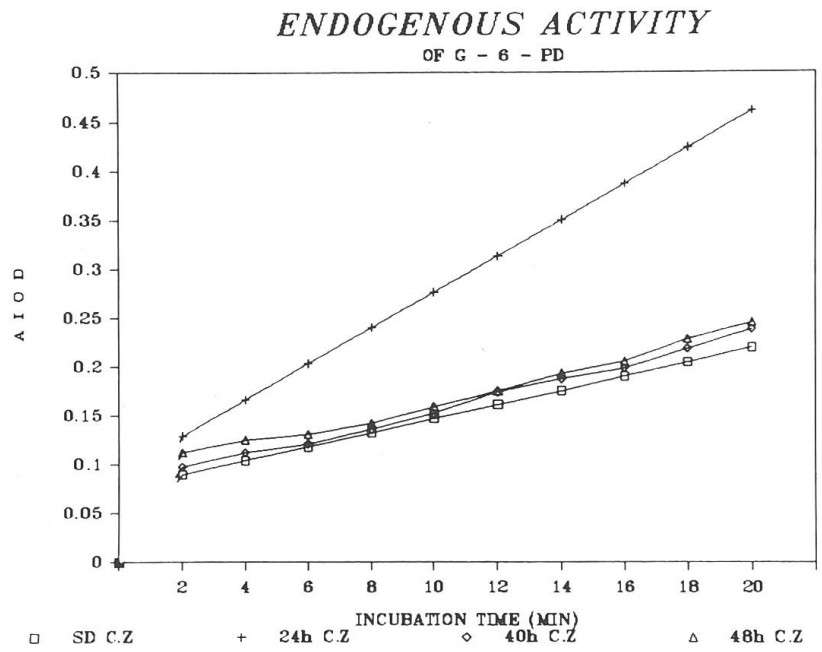

Fig. 6. – G-6-PD endogenous activity graph of absolute optical density/ μm^2 of reacted cytoplasm (AIOD) vs. time of incubation at 26°C in the central zone.
CZ, central zone; **SD,** short day apices; **24 h,** apices transferred to 24 h long-day length; **40 h,** apices transferred to 40 h long-day length; **48 h,** apices transferred to 48 h long-day length.

level and hence a greater flux through the PPP [10, 11]. This increased rate could be attributed to the vacuolization and elongation of cells, increased in the meristem size and the increased number of mitosis (Table 1 and Fig. 1), as well as an increasing number of nuclei synthesizing DNA [1, 12].

There is more carbohydrate imported into the apex [13] and may be oxidized by glycolysis or the oxidative PPP. It was reported [14] that one of the physiological changes associated with floral transition in photoperiodic-cold requiring plants is an increase of the soluble carbohydrate concentration in the apical bud. It was also shown [15] that there was an increase of the sucrose concentration and soluble glycosyl-equivalents concentrations in the evoked meristem of *Sinapis alba* by 50% of the control value.

The increase of G-6-PD activity obtained here occurred when the mitotic activity increased in both central zone after 24 h long-day and in the lateral zone after 40 h long-day. This mitotic activity became more important after 48 h long-day in the lateral zone to assume the preparation of a flower bud initiation. The changes in G-6-PD activity appeared to correspond to the changes in the apical cytology. Carbohydrate availability controls the cell's progression through the mitotic cycle in the meristematic zones of cultured roots [16]. Consequently, the data reported here show that both glucose-6-phosphate and mitotic activities in the vegetative and induced shoot apices of *S. oleracea* gave a zonation pattern and they were not symmetrically distributed within the induced apices. The rate of endogenous activity of G-6-PD indicates that the zonation pattern of the meristem could be related to the concentration of glucose (fructose) in the individual zones.

We assume that the distribution of the soluble carbohydrate concentration is the same within the various regions of the vegetative apex (short day apex). While this concentration becomes more important in the central zone of the induced apex, and it remains low in the lateral zones.

That is leads us to consider a polar distribution of the soluble carbohydrate which is localized in the central zone of the induced apex by 24 h long-day but disappears by 40 h long-day.

These results enhence the hypothesis that respiratory rates increase in the apical meristem during the induction of flowering.

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