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Inflorescence bud induction in *Vitis vinifera* L. cv. Thompson Seedless: Cytohistological events and starch accumulation in the shoot apex

by

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Induktion von Infloreszenzen in Knospen der Sorte Sultanina (*Vitis vinifera* L.): Cytologisch-histologische Veränderungen und Stärkeeinlagerung im Vegetationskegel

Zusammenfassung: An Knospen der Sorte Sultanina (*Vitis vinifera* L.) wurden Schnittuntersuchungen durchgeführt, um die cytologisch-histologischen Veränderungen während des Übergangs zur Blütenbildung aufzuzeigen. Messungen erbrachten eine Größenzunahme der Zellen, Zellkerne und Nukleolen sowie eine Zunahme der Zellwanddicke im Vegetationskegel und den Anlagen ruhender Knospen verglichen mit den entsprechenden Strukturen vegetativer Endknospen. Diese Veränderungen wurden ab der Knospe in Position 2 am Trieb (Zählung von apikal her) festgestellt. Sowohl Vegetationskegel als auch Anlagen ruhender Knospen zeigen Stärkeeinlagerung.

Key words: bud, inflorescence, induction, differentiation, histology, cytology, starch.

Introduction

The first cytohistological changes of vegetative apices in their transition to floral stage most mentioned in the literature, correspond to an increase in the mitotic index (BERNIER *et al.* 1967; BERNIER 1971; BODSON 1975), with synchronization at a cellular cycle stage (FRANCIS and LYNDON 1978), increase in the cellular, cytoplasmic, nucleolar (HAVELANGE 1980) and nuclear sizes (BERNIER *et al.* 1985) of cells in the peripheric and central zones of the apical meristem, followed by cell vacuolation and elongation in some studied species (JACQMARD *et al.* 1976). The overall vacuole apparatus size does not vary in other species (HAVELANGE *et al.* 1974). A heavy starch accumulation has also been observed in all apex zones together with these cytohistological changes (BERNIER 1971; BODSON 1975).

The grapevine (*Vitis vinifera*) is considered among the special cases where flowers are exclusively formed by specialized axillary meristems or latent buds (BERNIER *et al.* 1985). This bud inflorescence is formed by the development of an extralateral meristem called 'anlage' (SRINIVASAN and MULLINS 1976, 1980 a, 1980 b), a structure also present in *Parthenocissus inserta*, which begins its development by cell divisons of the peripherical zone and protoderm, giving rise to a spherical body perpendicular to the apex axis (MILLINGTON 1966). It can initiate tendrils, inflorescences or sprouts (PRATT 1974; SRINIVASAN and MULLINS 1981).

The few studies conducted on grapevines about changes occurring when going from vegetative to floral bud refer to the presence of a great number of starch granules

in latent buds during the inflorescence primordium formation (SRINIVASAN and MULLINS 1976) and to the DNA content per nucleus. It is mentioned that the 2C and 4C nucleus proportion varies in induced buds as to vegetative buds (SRINIVASAN and MULLINS 1980 b).

This research was conducted to verify if cytohistological changes, described for most species presenting simple buds, also occur on *Vitis vinifera* cv. Sultanina, with mixed buds and specialized axillary meristems. Besides, it was aimed to determine the initiation of floral induction in this variety growing in Chile under the IV Region conditions (Vicuña) as this area has become highly important in the cultivation of grapevine during the last years.

Materials and methods

Three bud samplings from *Vitis vinifera* cv. Sultanina plants, 6-year-old and growing in the IV Region (Chile) — 30° 2' S and 70° 45' W — were performed.

The first sampling was made in spring (November) selecting buds from 12 canes taken from different plants. The apical bud and the following latent bud sequence in each cane were fixed, counting from the apex towards the base: buds 1, 2, 3, 4, 5, 6, 8, 10, 13, 16 and 19. The second sampling was performed in fall (April) similar to the previous, but also removing from 4 different canes, buds 2, 4, 6, 8, 10, 12 and 14 counting from the base towards the apex. The objective of this sampling was to determine the occurrence of developing floral primordia. A third sampling made in early spring of the following year (August, September) consisted in removing every 3—5 d the buds appearing on alternate nodes from the apical bud towards the base. Thus, the buds located in apical position and on nodes 2, 4, 6, 8 and 10 along the developing shoot were fixed. The objective was to determine the time of floral induction, based on cytohistological traits of the apical meristem and the anlage.

The apical bud sampling allowed to have a typical vegetative apex to determine the cell changes when comparing them to latent bud apices since the flower induction is very early in this species (PALMA and JACKSON 1981; KLIEWER 1984). The buds were fixed with FAA (formalin, acetic acid, 70 % alcohol 5:5:90 v/v) and were dehydrated in the alcohol series up to 95 % alcohol. Later they were embedded in JB-4 plastic (Polyscience, Inc.) and sectioned at 2—4 µm with a Sorvall rotatory ultramicrotome. The sections were stained with Schiff reagent and 1 % aniline blue-black in 7 % acetic acid v/v and mounted in Canada balsam. Among 5—10 buds on each cane position were observed under a light microscope.

The relative size of anlagen originated from apical buds (directed to tendril) as well as of those from latent buds (probably directed to cluster primordia) presenting equal developmental stage was determined by measuring their width and height.

Cell size, nucleus and nucleolus diameters of terminal and latent bud cells as well as the anlagen cells (from latent and apical buds) were measured using amplified microphotos.

The mean values presented for apical buds correspond to measurements performed on 10 different ones. For latent buds, the values are derived from 4 different buds for each of 5 shoot positions. Mean values of each anlage type correspond to measurements carried out on 6 different anlagen.

The relative starch content of each structure (apical meristem and anlagen) was qualitatively determined by simple visual estimation. It ranked from 'without apparent starch' to 'very abundant' according to granules found in the different meristem zones.

Results

Fig. 1 shows the general aspect of the shoot apical meristem from *Vitis vinifera* cv. Sultanina with anlage development directed to tendril formation. As in this species induction is very early (PALMA and JACKSON 1981; KLIEWER 1984), latent buds below the apical bud should exhibit cell characteristics indicating the beginning of induction.

The cytological study proves that mean values of cell, nucleus and nucleolus diameters from shoot apical meristems are lower than those from latent buds, only cell and nucleus sizes being significantly different (Table 1 and Figs. 1—4).

Table 1

Diameter of cells, nuclei and nucleoli of shoot meristems and anlagen from apical and latent buds (μm)

Durchmesser der Zellen, Zellkerne und Nukleolen der Sproßmeristeme und Anlagen aus apikalen und ruhenden Knospen (μm)

	Apical bud	Latent bud	$\alpha = 0.05$
	Shoot meristem	Shoot meristem	
Cell	9.76	11.26	*
Nucleus	4.88	7.72	*
Nucleolus	2.28	2.43	N.S.
	Anlage	Anlage	
Cell	7.81	11.00	*
Nucleus	4.69	6.45	*
Nucleolus	1.50	2.46	*

* = Significant difference at 0.05 level (Student's T test).
N.S. = No significant difference at 0.05 level (Student's T test).

Cell diameter on latent buds is 13 % higher than on shoot apicals. Nucleus and nucleolus diameters are 58 and 8.5 % higher, respectively. Even though nucleus size is much greater than the indicated average in several latent bud apices, this value decreased when averaging it with some latent buds, located very near to the shoot apical meristem, in nodes 2 or 4, which presented smaller cells. These could probably not have been induced or just starting the process, still keeping the vegetative meristem cell characteristics. It is important to emphasize that besides these changes the general aspect of the meristematic tissue in apical buds (vegetative) is different from that of latent buds (hypothetically induced), showing a more defined tissue, very well delimited cells and a great cell distinctness owing to a greater cell wall thickness (Figs. 3 and 4).

As to anlagen, the differences in cell, nucleus and nucleolus sizes are even greater than already described for the apical meristems, all being significantly different (Table 1).

In anlagen from latent buds, cell diameter is 41 % greater than in those originated from vegetative apical buds which will give rise to tendrils. Also nuclear and nucleolar diameters are 37.5 % and 63.6 % greater, respectively. The same characteristics of thicker cell walls and cell distinctness in every area of the already described structure for latent bud apexes is also found in latent bud anlagen (Figs. 5 and 6). Anlagen width

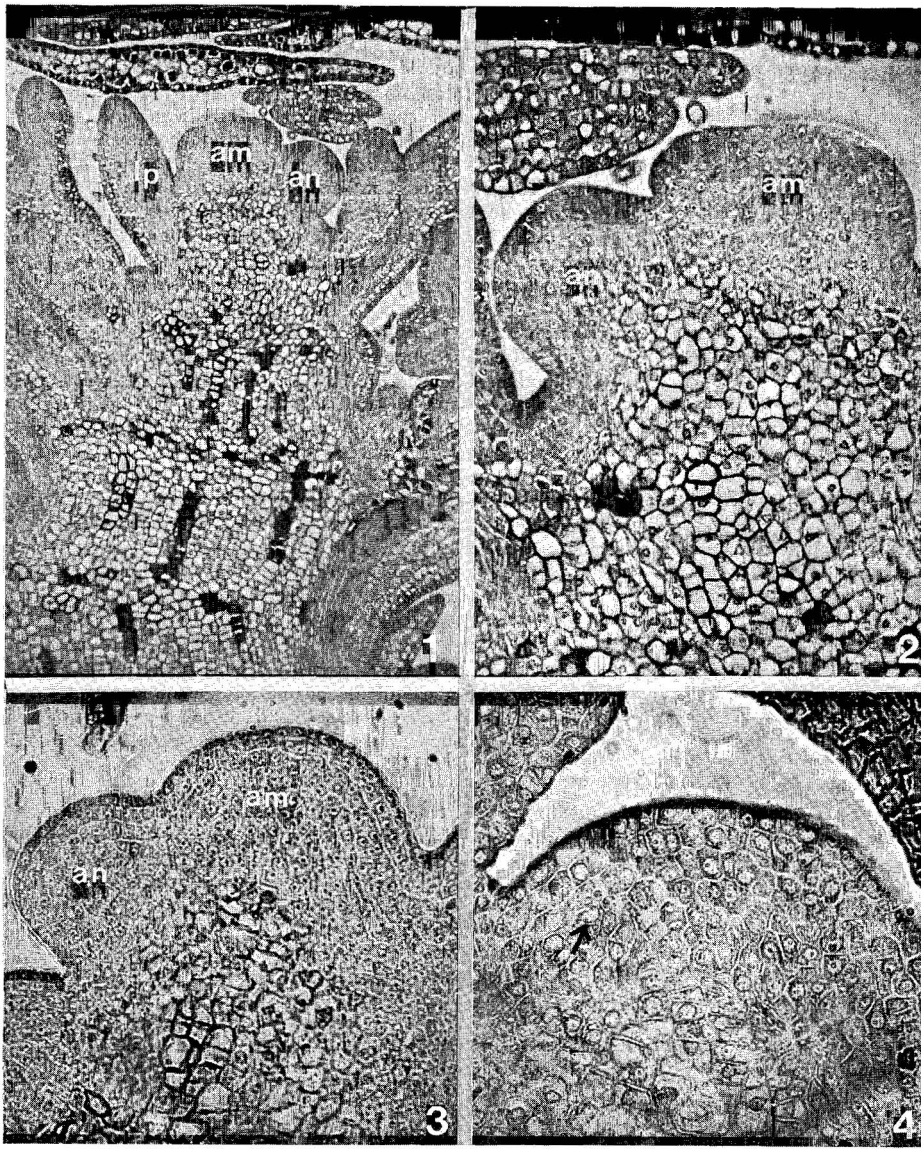

Fig. 1: General aspect of vegetative terminal bud showing anlage directed to tendril formation. x 107.
Fig. 2: Histological details of vegetative apex showing apical meristem and anlage. x 268.
Figs. 3 and 4: Histological detail of latent buds in node position 8 and 2, respectively. Starch is prewent in apex fo Fig. 4 (arrow). × 268 and × 428.

am = apical meristem, an = anlage, lp = leaf primordium.

Abb. 1: Übersichtsbild der vegetativen Terminalknospe mit prospektiver Rankenanlage. x 107.
Abb. 2: Histologische Details des Vegetationskegels mit Apikalmeristem und Anlage. x 268.
Abb. 3 und 4: Histologische Details ruhender Knospen in Position 8 bzw. 2. Im Vegetationskegel von 4 ist Stärke vorhanden (Pfeil). × 268 und × 428.

am = Apikalmeristem, an = Anlage, lp = Blattprimordium.

and height measurements also exhibit variations, the average width of apical bud anlagen being lower than those from latent buds, though not statistically different. The average height, however, is less in these latter (Table 2) and it proves very variable in the different structures observed.

Table 2

Mean size of anlagen formed from apical and latent buds (μm)

Durchschnittliche Größe der in apikalen und ruhenden Knospen entstandenen Anlagen (μm)

	Apical bud	Latent bud	$\alpha = 0.05$
Width	121.10	153.34	N.S.
Height	105.60	98.98	N.S.
W/H	0.87	0.63	N.S.

N.S. = No significant difference at 0.05 level (Student's T test).

The histological sections of latent buds apices and anlagen proved the occurrence of starch granules in most of them (Figs. 4—6). Starch is present in different meristem zones, being more abundant in the rib meristem (Tables 3 and 4). However, starch is not detected in any shoot meristems nor in anlagen of apical buds (Figs. 2—5).

Table 3

Relative starch accumulation in latent buds of different position on shoot and at different sampling dates

Relative Stärkeeinlagerung in ruhenden Knospen in unterschiedlicher Position am Trieb und zu verschiedenen Probenahmeterminen

Bud position	Date	Central zone (CZ)	Limit of CZ and RM	Rib meristem (RM)
2	28 August	+ + + +	−	+ + + +
	2 Sept.	+ + +	−	+ + + +
	6 Sept.	−	−	−
	9 Sept.	+	−	+ + + + +
	12 Sept.	−	−	+ +
4	2 Sept.	−	+	+ + + +
	6 Sept.	−	+ + + +	+ + + +
	9 Sept.	+ + + +	−	+ + + +
	12 Sept.	+	−	+ + + +
6	6 Sept.	+ + +	−	+ + + +
	9 Sept.	+	+ +	+ + + +
	12 Sept.	−	+ +	+ + +
8	9 Sept.	−	−	+ + + +
	12 Sept.	−	+ + + +	+ + + + +
10	12 Sept.	−	−	+ + + + +

Observations rank from 'without apparent starch' (−) to 'very abundant' (+ + + + +).

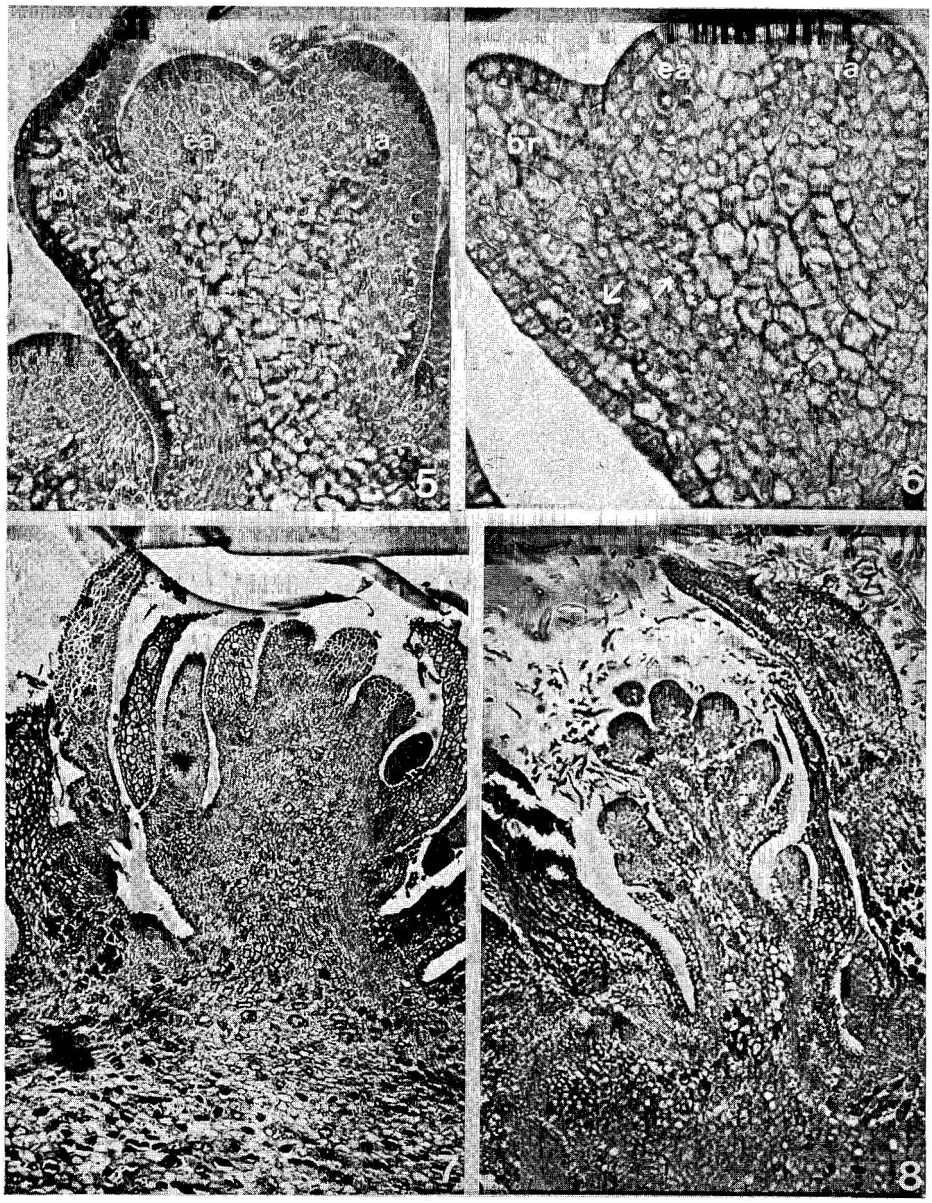

Fig. 5: Anlage from terminal vegetative bud showing initiation of internal and external arms and well developed bract with small cells, nuclei and nucleoli. x 428.

Fig. 6: Anlage from latent bud in node position 10 showing increase in cell, nucleus and nucleolus sizes and a clear delimitation of cells. Starch is present (arrows). x 428.

Figs. 7 and 8: Inflorescence primordia found in latent buds in node position 10. x 34 and 30.

br = bract, ea = external arm, ia = internal arm.

Table 4

Relative starch content in anlagen at different developmental stages

Relative Stärkeeinlagerung in Anlagen unterschiedlichen Entwicklungszustandes

Bud	Date	Anlage development stage	Central zone (CZ)	Limit of CZ and RM	Rib meristem (RM)
4	30 Aug.	Beginning[1])	–	–	+
4	17 Sept.	Beginning	–	–	+
8	17 Sept.	Beginning	–	–	+ + + +
8	9 Sept.	Beginning	–	–	+ + + +
6	3 Sept.	Spherical[2])	–	+ + +	+ + + +
4	6 Sept.	Spherical	–	–	+ +
6	6 Sept.	Spherical	+ + + +	–	+ + + +
8	10 Sept.	Spherical	–	–	+ +
10	17 Sept.	Arms[3])	–	–	+ + + +
8	12 Sept.	Arms	–	+ + + +	+ + + + +
10	12 Sept.	Arms	–	–	+ + + + +

[1]) Beginning of anlage formation.
[2]) Spherical structure perpendicular to rib meristem.
[3]) Anlagen with already formed arms.
Observations rank from 'without apparent starch' (–) to 'very abundant' (+ + + + +).

Discussion

The increase in cell, nucleus and nucleolus size of induced apices has already been mentioned by different authors for other species presenting a simple bud type (HAVELANGE 1980; BERNIER *et al.* 1985). The results of this study allow to add *Vitis vinifera*, a mixed-bud species, to the list of plants presenting these characteristics during floral process. Nevertheless, the apparent increase of wall thickness and the clear delimitation of apex and anlagen cells observed in induced latent buds in this research have never been mentioned for other species. It must be added that this characteristic could only be observed in amplified microphotos.

Starch content seems to be a highly important indicator of an induction process since the latent buds presenting more starch were also those exhibiting an increase in cell, nucleus and nucleolus diameters. As to this, SRINIVASAN and MULLINS (1976) have described the occurrence of a great number of starch granules and ^{14}C in latent buds during inflorescence primordium formation. This would indicate that the carbohydrate

Abb. 5: Anlage aus einer vegetativen Terminalknospe mit beginnender Differenzierung der inneren und äußeren Arme wohl entwickelter Knospenschuppen mit kleinen Zellen, Zellkernen und Nucleoli. x 428.

Abb. 6: Anlage aus ruhender Knospe in Position 10. Die Zellen, Zellkerne und Nucleoli haben sich vergrößert, und die Zellen sind deutlich gegeneinander abgegrenzt. Es ist Stärke vorhanden (Pfeile). x 428.

Abb. 7 und 8: Infloreszenzprimordien in ruhenden Knospen, Position 10. x 34 und 30.

br = Knospenschuppe, ea = äußerer Arm, ia = innerer Arm.

presence is influencing the floral process. LAVEE *et al.* (1967) also mentioned the carbohydrate accumulation as a condition for floral differentiation.

The presence of starch in the central zone below the apex top has also been observed in other plant species (KNOX and EVANS 1966) as well as in other apex areas during floral induction (BERNIER 1971; BODSON 1975). It has been suggested that the high carbohydrate level induces especially mitochondria growth and multiplication (BERNIER 1971) and there is a respiration increase during the floral induction process. As to the nutritional diversion hypothesis, induction causes the activation of the central zone, a requirement for floral induction and development through great nutritional requirements (assimilates) (SACHS 1977). Considering the data presented, starch can be supposed to be a determining aspect both for induction and during floral differentiation of this species. Besides, it can be concluded that floral induction begins immediately after the 1st node separates from the shoot apical bud considering the previously mentioned changes in cell, nucleus and nucleolus sizes, cell wall thickness and starch content observed in latent buds from nodal position 2 (from apex towards the base) and in the different anlage stages from buds in nodal position 4. This agrees with KLIEWER (1964) who mentioned manifestations that floral induction in this species occurs during the first weeks after the node separates from the apex. On the other hand, PALMA and JACKSON (1981) found a positive correlation of maximum temperature on floral induction when there were 3 visible nodes above the bud. As to floral differentiation, cytological changes and starch accumulation were observed in some anlagen from latent buds at node 4, which continue developing until they reach the characteristic form, with bract and both arms, in buds at position 8 and 10. It can be deduced, then, that these anlagen had originated from induced latent buds and were tending to cluster formation. Besides, cluster primordia were found in some buds in node position 10 (Figs. 7 and 8) during April and November samplings. Concurring with these conclusions, GIL *et al.* (1982) indicate that inflorescence primordia appear when there are approximately 10 leaves in the bud.

Summary

Shoot meristems and anlagen from latent buds in *Vitis vinifera* show an increase in cell, nucleus and nucleolus diameter. There is also an increase in cell wall thickness, clear cell delimitation of the tissues and starch accumulation in different zones of these structures.

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