

Flowering in *Vitis*: Effects of genotype on cytokinin-induced conversion of tendrils into inflorescences

by

C. SRINIVASAN and M. G. MULLINS

Die Blütenbildung bei der Rebe: Der Einfluß des Genotyps auf die Cytokinin-induzierte Umwandlung von Ranken in Infloreszenzen

Zusammenfassung. — Die Ranken verschiedener *Vitis*-Arten und -Bastarde entwickelten sich zu Infloreszenzen, wenn die Triebspitzen wiederholt mit 6-(Benzylamino)-9-(2-tetrahydropyranyl)-9H-purin (PBA; 50–1000 μM) behandelt wurden. Die Ranken männlicher Reben ließen sich durch PBA-Behandlung leichter in Infloreszenzen umwandeln als diejenigen weiblicher oder zwittriger Reben. *V. vinifera*, *V. rupestris* und ihre Kreuzungen reagierten stärker auf die PBA-Behandlung als *V. monticola*, *V. girdiana* und *Muscadinia rotundifolia*. Trauben mit lebensfähigen Samen entstanden aus rankenbürtigen Infloreszenzen der *V. vinifera*-Sorten Muskat von Alexandria, (♀) und Katakourgan (♂) sowie einer *V. vinifera* × *V. rupestris*-Kreuzung (♂).

Introduction

Flowering in the grapevine is regulated by the interaction of gibberellin and cytokinin. Gibberellin is involved in the formation of the inflorescence axis, the so-called anlage, and in the elongation of anlagen to form the tendrils. The formation of the inflorescence primordium from the anlage and the differentiation of flowers from the inflorescence primordium are both cytokinin-controlled processes (SRINIVASAN and MULLINS 1976, 1978, 1979, 1980).

In nature, grapevines produce numerous anlagen but most grow into tendrils and only a few anlagen give rise to inflorescences. This suggests that gibberellin is available for initiation of anlagen and for elongation of tendrils and that inflorescence formation is normally limited by the cytokinin supply. This concept is supported by the finding that tendrils and anlagen can readily be converted into inflorescences, both *in vivo* and *in vitro*, by treatment with exogenous cytokinin (SRINIVASAN and MULLINS 1978, 1979).

In the present paper we report on differences among *Vitis* species, hybrids and *V. vinifera* cultivars in the ease with which tendrils are converted into inflorescences by cytokinin.

Materials and methods

Plants of various *Vitis* species, hybrids and cultivars were propagated from rooted hardwood cuttings as described previously (SRINIVASAN and MULLINS 1976). The plants were grown in 1- or 2-l plastic bags in controlled environment cabinets (16 h

illumination, 27 °C light period — 22 °C dark period). Plants were illuminated with 16 fluorescent tubes (Cool white 183 cm, Sylvania, Seneca Falls, N. Y., U.S.A.) and 8 incandescent lamps (60 W). The irradiance at the level of the shoot tips was 45 W·m⁻². The plants were fed daily with Hoagland No. 2 nutrient solution.

The cytokinin, 6-(benzylamino)-9-(2-tetrahydropyran-2-yl)-9H-purine (PBA) (Shell Development Co., Modesto, Ca., U.S.A.) was dissolved in 5 % isopropanol. PBA was applied to shoot apices or to whole plants using Tween 20 (0.1 %) as wetting agent. Further details of treatments are given with results.

Morphological aspects of cytokinin-induced conversion of tendrils into inflorescences were studied by scanning electron microscopy. Methods of specimen preparation were as described earlier (SRINIVASAN and MULLINS 1976). For anatomical work tissues were fixed in 3 % glutaraldehyde in phosphate buffer (pH 6.8) and were sectioned according to BERLYN and MIKSCH (1976).

Results

1. Effects of genotype

Shoot apices and young tendrils (< 7 mm in length) were treated daily with 30 µl PBA (50–1000 µM) for up to 3 weeks. All the cultivars of *V. vinifera* responded to cytokinin treatment by producing inflorescences from tendrils but the number of inflorescences per plant, number of flowers per inflorescence and number of ripe berries produced varied widely among genotypes (Table). Muscat of Alexandria was very responsive to cytokinin treatment and tendrils began to produce flower buds with PBA at 50 µM. Shiraz, Cabernet Sauvignon and Red Emperor gave well-differentiated inflorescences with PBA (250 µM) but of these cultivars only Red Emperor set fruit. Tendrils of Sultana and Sideritis required treatment with PBA at 500 µM for flower production but neither of these cultivars produced fruit. The female grapevines, Katakourgan and a Cabernet Sauvignon × Sultana hybrid, both gave inflorescences with PBA, but only Katakourgan gave well-developed bunches. In male vines the effect of applying PBA to tendrils was to induce the formation of hermaphrodite flowers. Male vines of *Vitis* species and interspecific hybrids were highly floriferous with PBA. Gloryvine, a male hybrid grapevine (*V. vinifera* × *V. rupestris*) produced well developed bunches from tendril-derived inflorescences. Seedlings of *V. cinerea*, *V. monticola* and *V. girdiana* gave very small inflorescences (2–4 flowers) which failed to set fruit. Similarly, the tendrils of *Muscadinia rotundifolia*¹ gave small, unfruitful inflorescences when treated with PBA. *V. champini* (cv. Salt Creek, syn. Ramsay), *V. berlandieri*, *V. longii* and *V. cordifolia* did not respond to PBA and no flowers were formed when the cytokinin was applied either to shoot apices or as a whole-plant spray.

2. Mode of inflorescence development from tendrils

Tendrils which are < 7 mm in length and which have highly meristematic tips are responsive to cytokinin and give rise to flowers (Fig. 1). Tendrils which developed ridges and which have become flattened at their tips did not respond to PBA (Fig. 2).

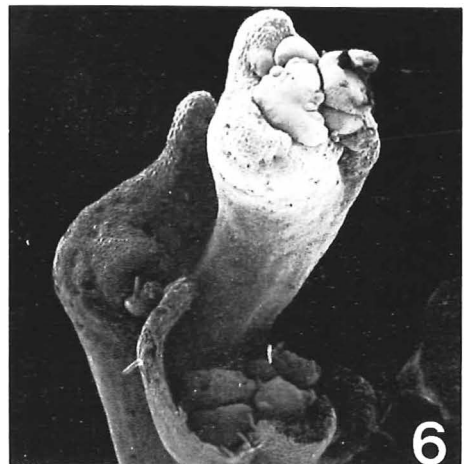
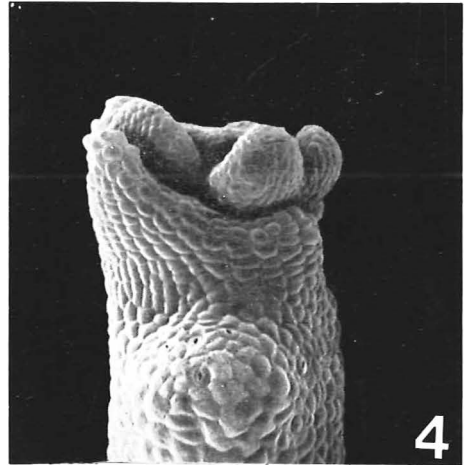
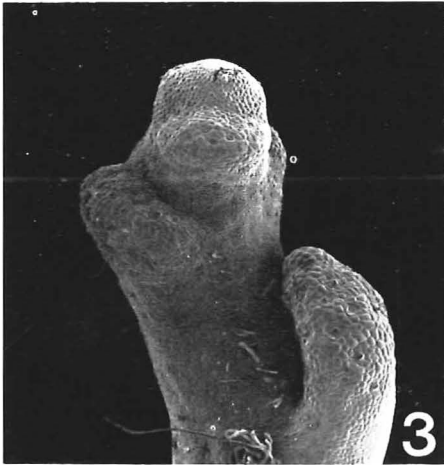
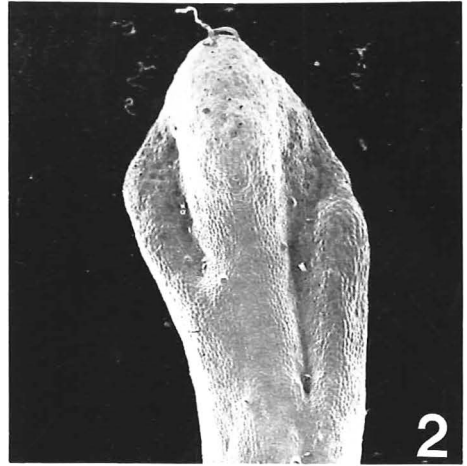
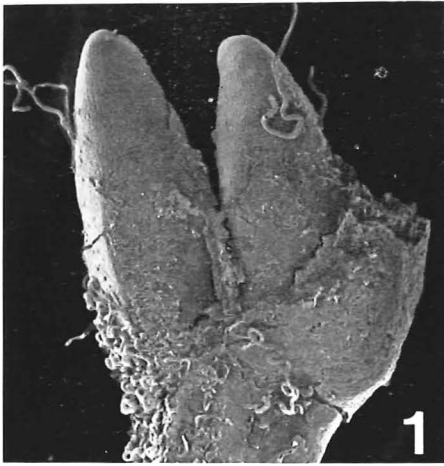
¹ The genus "*Muscadinia*" is used in accordance with OLMO, H. P. In: SIMMONS, N. W. (Ed.), 1976: Evolution of crop plants, pp. 294–298; Longman, London.

Effect of PBA on the conversion of tendrils into inflorescences and bunches of grapes in *Vitis* spp. and hybrids · General treatment: 250 μM PBA; treatments marked by an asterisk: 500—1000 μM PBA applied to shoot apices

Einfluß von PBA auf die Umwandlung von Ranken in Infloreszenzen und Trauben bei *Vitis*-Arten und -Kreuzungen · Allgemeine Behandlung: 250 μM PBA; durch Sternchen gekennzeichnete Behandlungen: 500—1000 μM PBA auf die Triebspitzen appliziert

Genotype	Number of plants treated	Mean number of inflorescences per plant	Mean number of flowers per inflorescence	Mean number of berries per plant
<i>Vitis vinifera</i>				
Hermaphrodite cultivars				
Muscat of Alexandria	10	6	88	81
Shiraz	5	3	29	—
Cabernet Sauvignon	10	3	21	—
Red Emperor	3	4	38	18
* Sultana	3	4	51	—
* Sideritis	3	2	53	—
Female vines				
* Katakourgan	2	5	59	63
* Cabernet Sauvignon \times Sultana	3	2	21	—
Hybrid vines				
(<i>V. rupestris</i> \times <i>cinerea</i>) \times <i>V. vinifera</i> (δ)	4	10	72	—
Gloryvine (<i>V. vinifera</i> \times <i>V. rupestris</i>) (δ)	10	8	171	146
(Grenache \times Cabernet Sauvignon) \times Gloryvine (δ)	1	58	98	—
JS 23416 (a complex hybrid (δ))	4	3	12	8
<i>Vitis</i> spp.				
Cuttings				
<i>Vitis rupestris</i> (δ)	4	5	189	—
<i>Vitis cinerea</i> (δ)	5	2	88	—
<i>Vitis cinerea</i> (δ)	3	2	18	—
Seedlings				
* <i>Vitis cinerea</i>	15	2	4	—
* <i>Vitis monticola</i>	1	6	3	—
* <i>Vitis girdiana</i>	1	1	2	—
<i>Muscadinia rotundifolia</i>	6	2.5	2	—

Branching of tendrils was the first sign of inflorescence formation (Fig. 3). If the apices of PBA-treated tendrils turned red, due to anthocyanin accumulation, before flower buds were visible these tendrils did not subsequently grow into inflorescences.



The site of flower formation differed with genotype. In vines which were the least responsive to PBA viz. *M. rotundifolia*, *V. monticola* and *V. girdiana*, one or more flowers appeared in a terminal or in a lateral position (Figs. 4 and 5). In *V. vinifera* cv. Muscat of Alexandria, three flower clusters were formed, one terminal and the other two in lateral positions (Fig. 6). This pattern was very common in tendrils which arose from the primary shoot of Muscat of Alexandria (Fig. 8).

Normal conical-shaped inflorescences were produced from tendrils in lateral shoots of male vines of *V. cinerea* (Fig. 9) and *V. rupestris*. In Katakourgan, a female vine, the shape of inflorescences differed from the normal configuration in that there were two distinct branches which often bore tendril-like appendages (Fig. 10).

Anatomical studies on the origin of flowers in cytokinin-treated tendrils indicated that there is no direct vascular connection between the newly-formed flower buds and the main axis of the tendril. Vascular connections are established initially between the tendril axis and the bract which subtends the emerging cluster (Fig. 7).

Fig. 1: A young tendril (approx. 5 mm in length). Photographed just before the commencement of cytokinin treatment, $\times 140$.

Fig. 2: A flat ridged tendril after 250 μM cytokinin treatment. This tendril will not produce an inflorescence. $\times 70$.

Fig. 3: A tendril with dichotomous branching in Muscat of Alexandria. This tendril will develop into an inflorescence with a daily supply of 30 μl PBA (250–1000 μM) for 3 weeks $\times 100$.

Fig. 4: Initiation of a single flower by the tip of the tendril in *M. rotundifolia*. This is a weak response to PBA. $\times 100$.

Fig. 5: Formation of a flower bud in a lateral position on a tendril of *V. monticola*. The flower bud is subtended by a bract. This is a weak response to PBA treatment. $\times 70$.

Fig. 6: Formation of three groups of flowers in a tendril of Muscat of Alexandria. The bract which covered the proximal group of flower initials has been removed to expose the flowers. $\times 40$.

Abb. 1: Junge Ranke von ca. 5 mm Länge, kurz vor dem Beginn der Cytokininbehandlung fotografiert. 140 \times .

Abb. 2: Flache, gefurchte Ranke nach Behandlung mit 250 μM Cytokinin. Aus einer so beschaffenen Ranke geht keine Infloreszenz hervor. 70 \times .

Abb. 3: Ranke mit dichotomer Verzweigung bei Muskat von Alexandria. Bei einer 3wöchigen Behandlung mit einer täglichen Dosis von 30 μl PBA (250–1000 μM) entwickelt sich diese Ranke zu einer Infloreszenz. 100 \times .

Abb. 4: Anlage einer einzelnen Blüte an der Spitze einer Ranke von *M. rotundifolia*. Die Reaktion auf PBA ist nur schwach. 100 \times .

Abb. 5: Bildung einer Blütenknospe in lateraler Position an einer Ranke von *V. monticola*. Die Knospe wird von einer Braktee gestützt. Hier erfolgte nur eine schwache Reaktion auf die PBA-Behandlung. 70 \times .

Abb. 6: Bildung von drei Gruppen von Blüten an einer Ranke von Muskat von Alexandria. Die Braktee, welche die proximale Gruppe der Blütenanlagen verdeckte, wurde entfernt, um diese sichtbar zu machen. 40 \times .

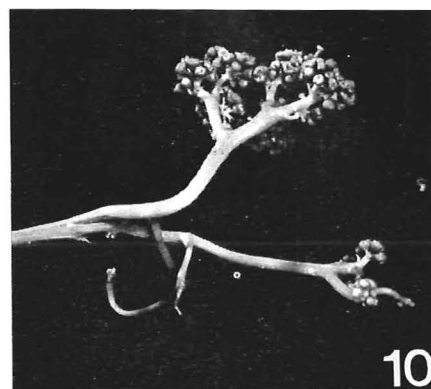
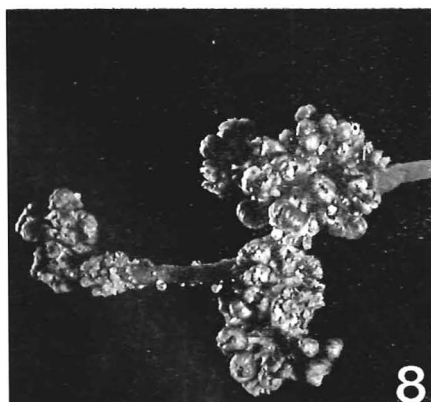
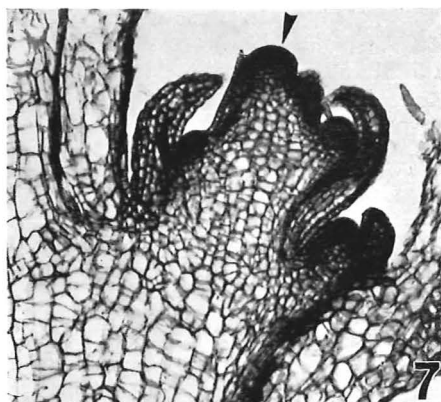


Fig. 7: A longitudinal section of a tendril showing the vascular connection between the flower initials and tendril. Note that the cells in the peripheral zone of the flower initials are highly meristematic (arrow). $\times 50$.

Fig. 8: Three groups of flowers on a tendril of Muscat of Alexandria (♀). This inflorescence developed from a tendril similar to the one shown in Fig. 6. $\times 1.2$.

Fig. 9: A normal conical-shaped inflorescence which developed from a tendril of a lateral shoot of *V. cinerea* (♂). $\times 0.8$.

Fig. 10: An inflorescence developed from a tendril of the primary shoot of Katakourgan, a ♀ *V. vinifera* grapevine. $\times 0.8$.

Abb. 7: Längsschnitt durch eine Ranke, der die Gefäßverbindung zwischen den Blütenanlagen und der Ranke zeigt. Zu beachten ist, daß die Zellen in der peripheren Zone der Blütenanlagen stark meristematisch sind (Pfeil). $50 \times$.

Abb. 8: Drei Gruppen von Blüten an einer Ranke von Muskat von Alexandria (♀). Diese Infloreszenz entwickelte sich aus einer Ranke, die der in Abb. 6 gezeigten ähnelt. $1,2 \times$.

Abb. 9: Normale kegelförmige Infloreszenz, die sich aus einer Ranke eines Geiztriebes von *V. cinerea* (♂) entwickelte. $0,8 \times$.

Abb. 10: Infloreszenz aus einer Ranke eines Haupttriebs von Katakourgan, einer ♀ *V. vinifera*-Sorte. $0,8 \times$.

Discussion

The present results and those reported earlier (SRINIVASAN and MULLINS 1978, 1979, 1980) strongly support the view that cytokinin is a principal regulator of flower formation in grapes. However, there are marked differences among genotypes in the intensity of flowering in response to cytokinin treatment of tendrils. Hermaphrodite cultivars of *V. vinifera*, male vines of *V. rupestris* and hybrid male vines of *V. vinifera* × *V. rupestris* were more susceptible to cytokinin-directed inflorescence formation than were other *Vitis* species and *M. rotundifolia*. Genetic differences in the flowering responses of plants to exogenous growth substances have been found also in herbaceous species. Some strains of *Silene armeria* are induced to flower by gibberellin application but other strains are gibberellin-insensitive (WELLENSIEK 1973).

Among the different sexual forms, male vines had a greater propensity to inflorescence production from tendrils than hermaphrodites and female vines. In many herbaceous and perennial plants male plants produce more flowers, or more numerous inflorescences, than other sexual forms (see review by LLOYD and WEBB 1977). However, cytokinin treatment in grapes favours the development of the pistil regardless of the original sex of the plant, be they male, female or hermaphrodite. In this regard NEGI and OLMO (1970) suggested that the cytokinin PBA counteracts a female sex inhibitor and thereby acts as a promoter of the development of ovary and ovules.

Cytokinins are involved in the control of many stages of reproduction in the grapevine including inflorescence formation (SRINIVASAN and MULLINS 1979), differentiation of flowers (MULLINS 1967), development of pistil in male flowers (NEGI and OLMO 1970), fruit set (WEAVER *et al.*) and somatic embryogenesis in unfertilized ovules of grapes *in vitro* (MULLINS and SRINIVASAN 1976). The mechanism of action of cytokinin in these phenomena, as in all other aspects of plant morphogenesis, has yet to be resolved.

Summary

Tendrils of several *Vitis* species and hybrids were transformed into inflorescences by repeated treatment of shoot apices with 6-(benzylamino)-9-(2-tetrahydropyran-9H-purine) (PBA, 50–1000 μM). Tendrils of male vines were more readily converted into inflorescences by PBA-treatment than those of females or hermaphrodite vines. *V. vinifera*, *V. rupestris* and their hybrids were more responsive to PBA-treatment than *V. monticola*, *V. girdiana* and *Muscadinia rotundifolia*. Bunches of grapes with viable seeds were produced by tendril-derived inflorescences of the *V. vinifera* cultivars Muscat of Alexandria (♂) and Katakourgan (♀) and of a *V. vinifera* × *V. rupestris* hybrid (♂).

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Prof. M. G. MULLINS
Department of Agronomy
and Horticultural Science
University of Sydney
Sydney, NSW 2006
Australia