Plant Physiol. (1978) 61, 127-130

Control of Flowering in the Grapevine (*Vitis vinifera* L.)

FORMATION OF INFLORESCENCES *IN VITRO* BY ISOLATED TENDRILS

Received for publication June 13, 1977 and in revised form August 23, 1977

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ABSTRACT

Tendrils produced from shoot tips of grapevine (*Vitis vinifera* L.) cultured *in vitro* on Nitsch's medium developed into inflorescences when 5 to 10 μM benzyladenine (BA) or 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) were applied directly to the tendril tips. Inflorescences did not form on tendrils if the cytokinins were supplied in the agar. Tendrils cultured in agitated liquid medium containing BA, PBA, or zeatin riboside showed profuse branching and tendrils were transformed into inflorescences. Calyx and corolla (calyptra) stamens and pistils developed normally in the presence of both zeatin riboside and PBA, but micro- and macrosporogenesis were absent.

Inflorescences were formed by tendrils from five cultivars (Muscat of Alexandria, Shiraz, Carbernet Sauvignon, Wortley Hall, and Sultana syn. Thomson Seedless) and also on tendrils from 12- to 15-week-old seedlings.

The physiology of flowering in photoperiodically controlled herbaceous plants has been the subject of intensive research (3, 4, 30) but there have been few studies on flower induction and differentiation in daylength-insensitive, woody perennials such as fruit trees and grapevines (2, 8). Research on flowering in most woody species is made difficult by the long juvenile or nonflowering periods of seed-grown plants, by the large size of adult trees, and by the annual occurrence of flowers. In addition, the reproductive anatomy of grapevines in particular is exceedingly complex and has only recently been clarified by scanning electron microscopy (27).

Flowering in grapes is a three-step process: (*a*) formation of anlagen; (*b*) differentiation of inflorescence primordia; and (*c*) formation of flowers. Anlagen are undifferentiated or uncommitted primordia which arise from terminal or axillary bud apices. Anlagen are formed in the current season and give rise either to inflorescence primordia or to tendril primordia. Usually, flowers are formed from inflorescence primordia at the time of bud burst in the following season (12, 21, 27).

Inflorescences and tendrils are both derived from anlagen and are homologous organs (1). Anlagen which undergo repeated branching give rise to inflorescences while those which produce only two or three branches give rise to tendrils (27). Accordingly, grapevine tendrils can be interpreted as weakly differentiated inflorescences. It follows that the control of inflorescence formation in grapes hinges upon the control of branching of anlagen or of tendrils.

The present paper is concerned with the nature of the stimuli which affect branching and this has been studied by growing isolated apices and tendrils in aseptic culture with various growth substances. Most experiments were carried out with explants from cuttings of grape cultivars but explants were also taken from open pollinated grape seedlings. In seedlings the first tendrils are formed at nodes 9 to 15, a few weeks after germination, but the first inflorescences may take 3 to 5 years to make their appearance in the field. In this paper we report on the formation of inflorescences and flowers *in vitro* from tendrils of grape cultivars and from the tendrils of 12- to 15-week-old seedlings.

MATERIALS AND METHODS

Rooted cuttings of the grapevine (cvs. Muscat of Alexandria, Shiraz, Cabernet Sauvignon, Wortley Hall, and Sultana syn. Thompson Seedless), were propagated from cool stored (4 C) canes as described previously (27). Grape seeds were extracted from winery marc and stratified (4 C) with perlite for at least 7 weeks before sowing.

Lateral (entre coeur) shoots from the regrowth from cuttings were collected and surface-sterilized by standard procedures. Shoot tips bearing one or two unfolded leaves were excised and cultured on agar (0.7%) with Nitsch's basal medium (18) supplemented with casein hydrolysate (0.1%) and various cytokinins (BA,[2] PBA, ZR,2iP kinetin, zeatin, and adenine). The tips were grown for 4 to 6 weeks (16-hr illumination, 3 wm^{-2} irradiance, 28 C) until tendrils were produced opposite to the newly formed leaves.

Explants consisting of tendrils alone, or of tendril plus associated leaves and axillary buds, were excised from the cultured shoot tips with the aid of a stereomicroscope. They were then grown in liquid culture supplemented with cytokinins as before, in a gyrorotatory incubator (80 oscillations min^{-1}, 16-hr illumination, 2.5 wm^{-2} irradiance, 28 C).

With open pollinated grape seedlings, the tips of primary shoots were removed from 12- to 15-week-old plants. These tips were cultivated *in vitro* to produce tendril explants and subsequently inflorescences, in the same way as from the tips of cultivars.

The tendril explants were then cultured for a further 6 to 9 weeks before being examined for inflorescence and flower formation using a scanning electron microscope. Preparation of scanning electron microscope specimens has been described (27). The inflorescences formed in gyrorotatory culture were irregular in shape, therefore dry wt and number of branches were taken as measures of size.

All cytokinins were obtained from the Sigma Chemical Co., except PBA which was a gift from the Shell Development Co.

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[2] Abbreviations: BA: N^6-benzyladenine; PBA: 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine; ZR: zeatin riboside; 2iP: 2-isopentenyladenine.

RESULTS

Effect of Cytokinins on Excised Shoot Tips Cultivated on Agar. Tendrils were formed by isolated shoot tips from grape cultivars after 4 to 6 weeks of culture on agar media containing casein hydrolysate. At this stage the tendrils comprised two branches which were enclosed by a bract (Fig. 1a).

If the medium contained BA or PBA (5–10 μM) numerous branches were produced at the tips of the tendrils, each branch being covered by a large overgrown bract (Fig. 1b). These branches were short lived and atrophied after a few days. Death of branches could not be prevented by addition of cytokinins to the agar. However, growth of branches could be sustained if the tips of tendrils were treated with frequent direct applications of BA or PBA: a fragment of lint moistened with BA or PBA (5–10 μM) was placed upon the tendril. Tendrils then continued to form branches and subsequently grew into inflorescences (Fig. 1b). These inflorescences were green or red. The presence of leaves or axillary buds did not affect the response of tendrils to the cytokinins.

Effect of Cytokinins on Inflorescence Formation on Tendril Explants in Liquid Medium. Culture of tendril explants with BA in liquid media, in a gyrorotatory incubator, resulted in prolific branching of tendrils and formation of inflorescences. Neither branching nor inflorescence formation was observed in the basal medium. With 2 to 10 μM BA there was a progressive increase in inflorescence size (Fig. 1, c and d). With increasing concentrations of BA there was a progressive increase in the number of branches and in the dry wt of inflorescences (Fig. 2, A and B). BA at a concentration of 15 μM was toxic. Other cytokinins which induced formation of inflorescences included PBA and ZR. Kinetin, 2iP, and zeatin failed to stimulate branching of tendrils. The response of tendrils to ZR was weak compared with the response to benzyl-substituted cytokinins and the optimum concentration of ZR (10 μM) was higher than that of the other cytokinins. Greatest growth of inflorescences was with BA and PBA (10 μM) (Fig. 2, A and B). Adenine was either without effect or was inhibitory to inflorescence formation.

Inflorescence Formation by Isolated Tendrils of Grapevine Seedlings. The tips of primary shoots of seedlings which had just begun to produce tendrils (*i.e.* after production of 9–15 nodes) were cultured on agar. Young tendrils were excised from cultured tips and were grown in liquid culture with the same procedures as used for explants from the cultivars. When these seedling tendrils were cultivated for 3 to 4 weeks with PBA (10 μM) there was prolific branching of the tendrils and formation of inflorescences. These inflorescences were mostly red.

DEVELOPMENTAL ANATOMY OF INFLORESCENCES AND FLOWERS PRODUCED BY ISOLATED TENDRILS

Inflorescences. The process of inflorescence formation *in vitro* is similar to the process *in vivo*. In culture the inflorescence arose by repeated branching of young tendrils (Fig. 1d). In the intact vine the primordial inflorescences arise by repeated branching of the anlage. In the cultured tendrils each branch was subtended by a large overgrown bract. Branches were usually pale green but the bracts were red. In some instances the bracts developed into leaves. After a period of profuse branching by isolated tendrils, groups of three to five flowers began to appear in the explants (Fig. 1e).

Flowers. The flower buds which developed on tendrils grown with BA or PBA formed calyx and corolla (calyptra in grapes) (Fig. 1e) but the androecium and gynoecium were rudimentary. Subculture of these underdeveloped inflorescences onto various basal media (15, 18, 29), supplemented with single cytokinins, failed to induce further growth of the androecium and gynoecium. However, when partly formed flowers or tendrils were grown with a sequence of cytokinins there was considerable further development of flower parts. For example, a small green inflorescence was formed with ZR (10 μM). If the medium was renewed after 3 weeks then supplemented with PBA (5 μM) as well as ZR (10 μM) it was found that the development of calyx and calyptra was complete and that stamens and pistils were also produced (Fig. 1, f and h). In some of these flowers the gynoecium was abnormal and there were instances of open carpels (Fig. 1g). So far, the anthers and ovules produced by the flowers induced on cultured tendrils have been empty and no evidence has been obtained of microspore or megaspore production *in vitro*.

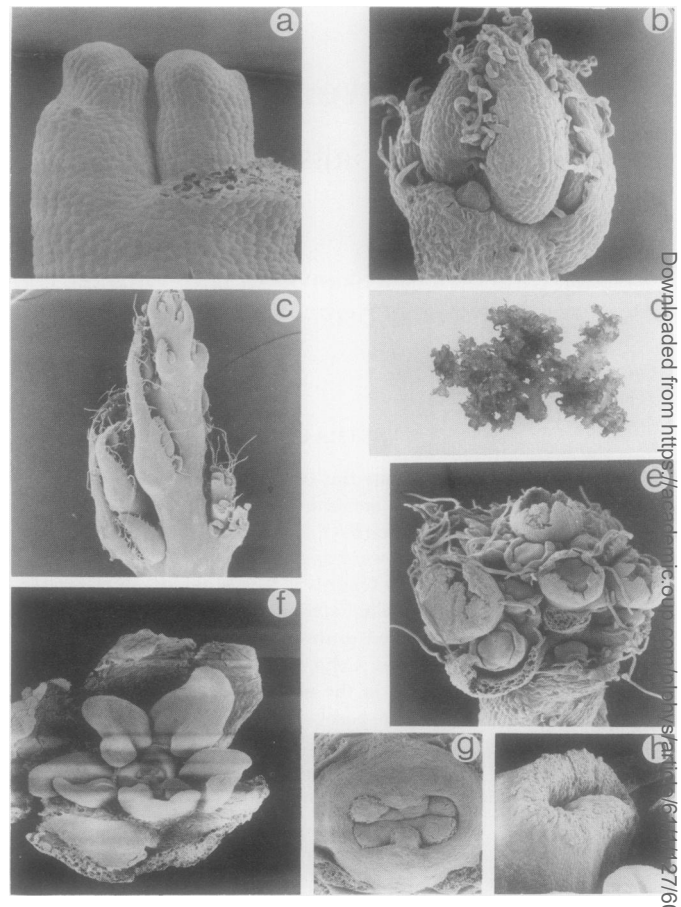

FIG. 1. Scanning electron micrographs (except Fig. 1d) of inflorescence and flower formation *in vitro* on grapevine tendrils. (a) A young tendril excised from a cultured shoot tip (× 200). (b) Inflorescence formation on one arm of the tendril, by direct application of 5 to 10 μM BA on tips of tendril growing on agar (× 75). (c) Inflorescence formation at 2 to 3 μM BA (low concentration) in liquid medium (× 30). Note linear growth. (d) Inflorescence formation at 5 to 10 μM (high concentration) BA (× 1). Note that inflorescence is broad rather than linear and has more branches. (e) Flower formation on one branch of inflorescence (× 50). Note formation of calyx and calyptra. (f) Fully developed flower showing the stamens and pistil (× 50). (g) Open development of carpel and ovules (× 90). (h) Style and stigma formation (× 150).

DISCUSSION

Flower induction and development in response to exogenous cytokinins have been observed in a few herbaceous plants (11, 13, 17). There is a marked increase in cytokinin activity in the ascending xylem sap during bud burst in some woody perennials (7, 10, 24) but direct involvement of cytokinins in inflorescence formation has not been reported. Nevertheless, cytokinins have

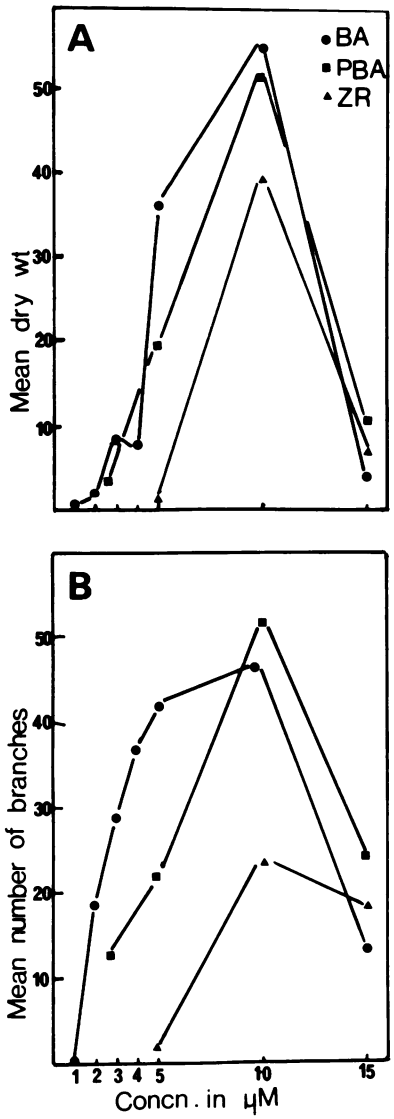

FIG. 2. Effect of cytokinins on mean dry wt (A) and mean number of branches (B) per inflorescence (mean of nine cultures). Cultures were grown with Nitsch's basal medium supplemented with 0.1% casein hydrolysate and 2% sucrose.

been shown to promote the growth of flowers in grapevine cuttings (14). In the present experiment, inflorescences and flowers have been made to grow on young grapevine tendrils by treatment with cytokinins.

Morphologically, the grapevine inflorescence is a system of branches (23), and it is clear that cytokinins are involved in the control of branching. Additional evidence of a relationship between branching of meristems and formation of inflorescences in response to cytokinins is to be found in herbaceous species (25). In *Chenopodium rubrum*, a short day plant, the appearance of flowers is preceded by profuse branching of the stem apex. With conditions of suboptimal induction there is production of branches but flowers are not formed (9). In grapes, it is noteworthy that low concentrations of BA and PBA caused branching of isolated tendrils but that formation of flowers required exogenous cytokinins at a concentration of 5 to 10 μM. In *Carex* spp., application of BA caused changes in the pattern of growth of the inflorescence; determinate branching was replaced by indeterminate branching (26). A similar response to exogenous cytokinins was found in our cultures of the grapevine (Fig. 1d), wherein cytokinin induced profuse lateral branching of tendrils which have been defined as determinate shoots (28). These observations, in which formation of inflorescences is closely associated with lateral branching, indicate that the mechanism of flower formation involves a weakening of apical dominance in the tendril. The role of cytokinin and of other growth substances in controlling apical dominance is well known (19). Effects of cytokinin on flowering in grapes are consistent with the view of Zeevaart (30) that flowering in woody perennials is governed by the response of apices to an interaction of growth substances, rather than by response to a specific florigen.

Morphogenetic effects of ZR and PBA have been indicated previously in the grapevine and include promotion of flower growth *in vitro* (20) and development of pistils by staminate flowers (16). ZR is the main cytokinin of the xylem sap of the grapevine (24). The requirement for both ZR and PBA for production of inflorescences and flowers in cultured tendrils is difficult to interpret. However, the importance of multiple forms of cytokinins in the control of growth and development has been emphasized, by some workers (6). Abnormalities such as multiple lobes on calyx and calyptra, open ovules, and short styles (Fig. 1, g and h), which were found in grape flowers in response to PBA, have also been reported in cytokinin-treated plants of *Browallia demissa* (5).

The absence of micro- and megasporogenesis in the flowers formed *in vitro* suggests that factors other than cytokinins, minerals and sucrose are necessary for these processes. Empty ovules lacking an egg apparatus have been reported previously in grape flowers grown *in vitro* with ZR (20) but pistil development was promoted by PBA in staminate flowers in intact plants (16). It seems that factors which trigger meiosis in mitotic cells are available in intact plants but lacking in the tissue culture media used for our explants.

The results of the present experiment confirm our earlier conception that the grapevine tendril is a weakly differentiated inflorescence (27). Accordingly, the appearance of the first tendril on a grape seedling is a manifestation of phase change; *i.e.* the transition from the juvenile nonflowering stage to the adult flowering condition and not merely a change in form associated with the acquisition of a climbing habit (22). This knowledge is being applied to the induction of precocious flowering for plant-breeding purposes.

Acknowledgment – We thank P. B. Goodwin for critical review of the manuscript.

LITERATURE CITED

1. BUGNON F, R BESSIS 1968 Biologie de la vigne. Acquisitions Recentes et Problèmes Actuels. Masson, Paris, pp 3-26
2. BUTTROSE MS 1969 Fruitfulness in grapevine: effects of light intensity and temperature. Bot Gaz 130: 166-173
3. CHAILAKHYAN MKH 1968 Internal factors of plant flowering. Annu Rev Plant Physiol 19: 1-36
4. EVANS LT 1971 Flower induction and the florigen concept. Annu Rev Plant Physiol 22: 365-394
5. GANAPATHY PS 1969 Floral morphogenesis and flowering in aseptic cultures of *Browallia demissa* L. Biol Plant 11: 65-74
6. HALL RH 1973 Cytokinins as a probe of developmental processes. Annu Rev Plant Physiol 24: 415-444
7. HEWETT EW, PF WAREING 1974 Cytokinin changes during chilling and bud burst in woody plants. *In* RL Bieleski, AR Ferguson, MM Cresswell, eds, Mechanism of Regulation of Plant Growth, Bulletin 12. Royal Society of New Zealand, Wellington, pp 693-701
8. JACKSON DI, GB SWEET 1972 Flower initiation in temperate woody plants. Hort Abstr 42: 9-24
9. KREKULE J, B HORAVKA 1972 The response of short day plant *Chenopodium rubrum* to ABA and GA treatment applied at two levels of photoperiodic induction. Biol Plant 14: 254-259
10. LUCKWILL LC, P WHYTE 1972 Hormones in the xylem sap of apple trees. SCI Monogr 31: 87-101
11. MAHESWARI SC, R VENKATARAMAN 1966 Induction of flowering in a duckweed *Wolfia microscopia* by a new kinin, zeatin. Planta 70: 304-306
12. MAY P 1964 Über die knospen-und infloreszenzentwicklung der Rebe. Wein-wiss 19: 457-485

13. MICHNIEWICZ M, A KAMIENSKA 1964 Flower formation induced by kinetin and vitamin E treatment in cold requiring plant (*Chicorium intybus* L.) grown under non-inductive conditions. Naturwissenchaften 51: 295-296
14. MULLINS MG 1967 Morphogenetic effects of roots and of some synthetic cytokinins in *Vitis vinifera* L. J Expt Bot 18: 206-214
15. MURASHIGE T, F SKOOG 1962 A revised medium for rapid growth and bioassay with plant tissue cultures. Physiol Plant 15: 473-497
16. NEGI SS, HP OLMO 1972 Certain embryological and biochemical aspects of cytokinin SD 8339 in converting sex of male *Vitis vinifera* (Sylvestris). Am J Bot 59: 851-857
17. NITSCH C 1968 Effect of growth substances on the induction of flowering of a short day plant *in vitro*. *In* F Wightman, F Setterfield, eds, Biochemistry and Physiology of Growth Substances. Runge Press, Ottawa Canada, pp 1385-1397
18. NITSCH JP, C NITSCH, S HAMON 1968 Realisation experimentale de l' "androgenese" chez divers *Nicotiana*. CR Soc Biol 162: 369-372
19. PHILIPS IDJ 1975 Apical dominance. Annu Rev Plant Physiol 26: 341-367
20. POOL RM 1975 Effect of cytokinin on *in vitro* development of concord flowers. Am J Enol Viticult 26: 43-46
21. PRATT C 1971 Reproductive anatomy of grapes—a review. Am J Enol Viticult 22: 92-109
22. PRATT C 1974 Vegetative anatomy of cultivated grapes—a review. Am J Enol Viticult 25: 131-150
23. RICKETT HW 1944 The classification of inflorescence. Bot Rev 10: 187-231
24. SKENE KGM 1972 Cytokinins in the xylem sap of grape vine canes: changes in activity during cold storage. Planta 104: 89-92
25. SKOOG F 1971 Aspects of growth factor interactions in morphogenesis of tobacco tissue cultures. *In* Les Cultures de Tissue de Plantes, No. 193. Coll Int CNRS, Paris, pp 115-135
26. SMITH DL 1969 The role of leaves and roots in the control of inflorescence development in *Carex*. Ann Bot 33: 505-514
27. SRINIVASAN C, MG MULLINS 1976 Reproductive anatomy of the grapevine (*Vitis vinifera* L.): origin and development of the anlage and its derivatives. Ann Bot 38: 1079-1084
28. TUCKER SC, LL HOEFERT 1968 Ontogeny of the tendril in *Vitis vinifera*. Am J Bot 55: 1110-1119
29. WHITE PR 1943 A Handbook of Plant Tissue Culture. Ronald Press, New York
30. ZEEVAART JAD 1976 Physiology of flower formation. Annu Rev Plant Physiol 27: 321-348