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Induction of precocious flowering in grapevine seedlings by growth regulators

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ABSTRACT

*Grapevine,
Induction to flower,
Growth substances, CCC,
Accelerating generations.*

Seedlings of *Vitis vinifera*, cvs Cabernet Sauvignon and Muscat Hamburg were induced to flower within 4 weeks of germination by repeated treatment of shoot apices with 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA, 250 μ M). Similarly, precocious flowering was induced by PBA in plantlets derived from somatic embryos of a *V. vinifera* \times *V. rupestris* hybrid. In cytokinin-treated plants tendrils were converted into inflorescences. Flower number per tendril increased with the age of the seedling. The number of fertile tendril per plant, and flower number per tendril were promoted by combined treatments of PBA (500 μ M) and chlormequat (CCC) (3 000 μ M). Mixtures of cytokinins and chlormequat induced inflorescence formation in the latent buds of 3-month-old seedlings. When these buds were induced to grow out by pruning they gave bunches with up to 200 ripe berries with highly germinable (80 p. 100) seed. The significance of these results for grape breeding is discussed.

RÉSUMÉ

*Vigne,
Mise à fleur,
Substances de crois-
sance, CCC,
Accélération des généra-
tions.*

Induction d'une mise à fleur précoce chez des plants de semis de vigne par des régulateurs de croissance

Sur des plants de semis de *Vitis vinifera* cultivars Cabernet Sauvignon et Muscat de Hambourg, la floraison a été provoquée à quatre semaines de la levée par des traitements répétés des extrémités de tiges avec de la 6-(benzylamino)-9-(2-tétrahydropyranyl)-9H-purine (PBA 250 μ M). De la même manière, on a induit avec le PBA une floraison précoce sur des plantules dérivées d'embryons somatiques d'un hybride *V. vinifera* \times *V. rupestris*. Sur les plantes traitées à la cytokinine, les vrilles ont été converties en inflorescences. Le nombre de fleurs par vrille augmente avec l'âge du plant de semis.

Le nombre de vrilles fertiles par plante et le nombre de fleurs par vrille ont été augmentés par des traitements combinés de PBA (500 μ M) et de chlormequat (CCC) (3 000 μ M). Des mélanges de cytokinines et de chlormequat ont induit la formation d'inflorescences dans les bourgeons latents de plants de semis âgés de 3 mois.

Quand ces bourgeons sont forcés en taillant la tige au-dessus d'eux, ils ont fourni des grappes avec jusqu'à 200 baies mûres portant des grains à haute faculté germinative (80 p. cent).

La signification de ces résultats pour l'amélioration génétique de la vigne est discutée.

INTRODUCTION

The achievements of grape breeders in the last 30 years show clearly that genetic improvement of grapevines, whether for production of wine, fresh fruit or dried fruit, is a feasible proposition and that new cultivars can be produced for a given environment which exceed the traditional cultivars in quality or other desirable characters. However, grape breeding is a long-term and labour-intensive operation. It has been estimated that the evaluation of a single seedling ties up 3-4 m² of viticultural land for 7 years (WAGNER & BRONNER, 1974). The expense of conventional fruit breeding programs has prompted interest in methods which speed-up the breeding process, including the acceleration of generations by induction of precocious flowering in seedlings.

Much research has been done in France on the intensification of vine breeding by use of hydroponic culture for induction of early fruiting in glasshouse-grown seedlings (HUGLIN & JULLIARD, 1964) and by use pre-selection (WAGNER, 1967, 1975; WAGNER & BRONNER, 1974). These two approaches are combined in the breeding strategy proposed by BOUQUET (1977).

The present report is concerned with induction of precocious flowering in grape seedlings by use of growth regulators. The techniques to be described here are based on our research on the hormonal control of flowering in grapevine cultivars (SRINIVASAN & MULLINS, 1976, 1978, 1979, 1980). In brief, the specialized axillary buds (latent buds) of adult grapevines produceanlagen, or « uncommitted » primordia, which give rise to tendril primordia, or to inflorescence primordia. Anlage formation is a gibberellin-

controlled process but the subsequent differentiation of Anlagen is regulated by the gibberellin : cytokinin balance. High levels of gibberellin favour the elongation of Anlagen and formation of tendrils primordia. High levels of cytokinin induce branching of Anlagen and the formation of inflorescence primordia. Tendril primordia and inflorescence primordia are homologous organs (they arise from the same Anlagen) and the one can be converted into the other by exogenous growth regulators. Tendrils are converted into inflorescences by treatment of the tendril apex with benzyladenine, with 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA), or with mixtures of cytokinin and growth retardants.

This paper describes some responses to PBA and growth retardants of open-pollinated seedlings of Cabernet Sauvignon and Muscat Hamburg and plantlets produced *in vitro* from somatic embryos of a *Vitis vinifera* × *Vitis rupestris* hybrid.

MATERIALS AND METHODS

Seeds of *Vitis vinifera* L., cvs Cabernet Sauvignon and Muscat Hamburg, were stratified (4 °C) in moist sand. After 6-8 weeks of chilling the seeds were germinated in sand. When the seedlings were 100-150 mm in height they were transplanted to a perlite : peat (4 : 1) mixture in 1.25 l plastic containers. Somatic embryos of the male hybrid (ornamental) grapevine, Gloryvine, (*Vitis vinifera* L × *V. rupestris* Scheele) were produced from the connective of cultured anthers by the method of RAJASEKARAN & MULLINS (1979). The embryos were chilled (4 °C) for 4 weeks, germinated in agar based basal medium (NITSCH and NITSCH, 1969) and then planted in the glasshouse in peat : perlite medium (1.3).

The seedlings of Cabernet Sauvignon and Muscat Hamburg and the plantlets of Gloryvine were irrigated daily with Hoagland No. 2 nutrient solution and grown in a controlled environment cabinet (27 °C day-22 °C night ; 16 h photoperiod, irradiance 40 Wm⁻²) from 16 cool-white fluorescent tubes (Sylvania, Seneca Falls, N.Y., U.S.A.).

Cytokinins and growth retardants were applied either as whole plant sprays or as droplets to shoot apices. The

Cytokinins and growth retardants were applied either as whole plant sprays or as droplets to shoot apices. The cytokinin 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) was dissolved in 5 p. 100 isopropanol (v/v). Benzyladenine (BA) was dissolved in 2 ml of 0.05 N HCl and diluted with water to 1 litre. The growth retardants (2-chloroethyl) trimethyl ammonium chloride (chlormequat), succinic acid 2-2-dimethyl hydrazide (SADH) and Ammonium (5-hydroxycarvacryl) trimethyl chloride piperidine carboxylate (ACPC) were dissolved in distilled water. Tween 20 (0.1 p. 100) was used as a wetting agent.

RESULTS

Treatment of shoot apices of 2 to 3 week-old seedlings of Cabernet Sauvignon with PBA (30 µl, 250 µM) caused inflorescences to be formed in place of tendrils. Formation of flower initials required daily application of PBA for 10 days. With this treatment even the first-formed tendril was transformed into an inflorescence (fig. 1A, B) and the seedlings bore flowers within four weeks of germination. The number of flowers produced by tendrils in response to PBA increased with the age of the seedling (table 1), as did the number of fruits which were borne to maturity. None of the flowers formed on one-month-old Cabernet seedlings set fruit. Older seedlings were more fruitful and at six

TABLE 1

Effect of age of Cabernet Sauvignon seedlings on the induction of precocious flowering (Mean of ten replicates) by PBA (250 µM)
Effet de l'âge de plants de semis de Cabernet Sauvignon sur l'induction d'une floraison précoce (Moyenne de 10 répétitions) par le PBA (250 µM)

Age of seedlings Age des plants de semis	Mean no. of tendrils per plant transformed into inflorescences Nombre moyen de vrilles transformées en inflo- rescences par plante ± SE	Mean no. of flowers per tendril Nombre moyen de fleurs par vrille ± SE
1 month	2.3 ± 0.45	8.4 ± 0.98
4 months	2.6 ± 0.16	13.9 ± 0.85
6 months	2.9 ± 0.30	18.4 ± 1.56

months of age the plants produced small bunches of up to a dozen ripe berries. The latent buds of these seedlings did not produce inflorescence primordia.

In three-week-old seedlings of Muscat Hamburg application of chlormequat, either as droplets to shoot apices or as sprays to whole plants, did not alter the normal course of tendril development. However, treatment of shoot apices with PBA droplets, or with mixtures of PBA plus chlormequat, caused the tendrils to form inflorescences. Similarly, spraying the seedlings with mixtures of PBA and chlormequat produced a few inflorescences.

An effective treatment to induce several tendrils on each plant to form inflorescences, and to increase the number of flowers per tendril, was to spray the seedlings with a mixture of PBA (500 µM) and chlormequat (3 000 µM) followed by topical treatment of shoot apices and young tendrils (< 5 mm long) with PBA (250 µM) (table 2). Spraying seedlings with ACPC (200 µM) considerably reduced shoot growth and tendril growth. Subsequent treatment of shoot apices and young tendrils with PBA (250 µM) caused the initiation of flowers from tendrils (table 2).

Mixtures of cytokinins (500 µM-1 000 µM PBA ; 1 000 µM BA) and growth retardants (chlormequat 3 000 µM ; SADH 1 250 µM ; ACPC 200 µM) were sprayed on 3-month-old seedlings of Muscat Hamburg. Examination of latent buds 3 months after the treatment i.e. 6 months after germination, showed that latent buds on nodes 18-30 bore 1 or 2 inflorescence primordia in all treated vines except those sprayed with PBA plus SADH (table 3). In addition, inflorescences were also produced from tendrils of primary and lateral shoots in vines treated with PBA or BA plus chlormequat (fig. 1C). Activation of latent buds of treated vines by pruning induced the inflorescence primordia to develop into normal inflorescences. Up to nine inflorescences per plant were formed in 6-7 month-old seedlings which received PBA or BA plus chlormequat treatments. These inflorescences produced 1 or 2 bunches of ripe grapes. The number of fruits per bunch varied from 46 to 207 (fig. 1E).

Shoot apices of plantlets grown from anther-derived somatic embryos of Gloryvine were fed daily with PBA (500 µM) for 1-3 weeks. Hermaphrodite flowers developed on the tendrils of plantlets which were only 4-5 weeks old, that is, in plants which had been grown for 4-5 weeks in non-sterile conditions following transfer from the culture vessels. However, normal-sized inflorescences and bunches of grapes were produced only in 4 to 6-month-old vines

TABLE 2

Influence of PBA and growth retardants on precocious flowering in Muscat Hamburg seedlings
Influence du PBA et de retardateurs de croissance sur la floraison précoce chez des plants de semis de Muscat de Hambourg

Treatments (*) Traitements	Mode of application Mode d'application	Mean no. of tendrils bearing flowers Nombre moyen de vrilles portant des fleurs ± SE	Mean no. of flowers per tendril Nombre moyen de fleurs par vrille ± SE
PBA (500 µM).....	whole plant spray	5.5 ± 0.2	9.8 ± 1.2
ACPC (200 µM).....	whole plant spray	3.0 ± 1.0	13.0 ± 3.6
Chlormequat (Chl) (3 000 µM).....	whole plant spray	3.3 ± 1.1	10.3 ± 3.2
PBA (250 µM).....	shoot tip only	1.3 ± 0.6	1.2 ± 0.7
PBA + Chl (250 µM) (3 000 µM).....	shoot tip only	3 ± 0.8	9.2 ± 2.5
PBA + Chl (500 µM) (3 000 µM).....	whole plant spray	6.5 ± 0.8	30.3 ± 9.6

(*) Shoot tips of all vines were treated with 30 µM PBA (250 µM) everyday for 10 days. Vines in which shoot tips were not treated with PBA did not flower irrespective of other treatments. Inflorescences were formed by tendrils of the primary shoot.

TABLE 3

Inflorescence formation by latent buds in 6-month-old seedlings of Muscat Hamburg which were sprayed with growth regulators 3 months after germination (mean of 5 replicates)
Formation d'inflorescences par des bourgeons latents chez des plants de semis de Muscat de Hambourg traités avec des régulateurs de croissance 3 mois après la levée (moyenne de 5 répétitions)

Treatments Traitements	Mean number of inflorescence- bearing buds per vine Nombre moyen de bourgeons contenant des inflorescences par plante	Mean number of inflorescence primordia per bud Nombre moyen d'ébauches d'inflorescence par bourgeon	Nodes at which fertile buds developed Nœud portant les bourgeons fertiles
Control.....	0	0	0
Chlormequat (Chl) (3 000 µM).....	1.2	1.0	24-30
PBA (1 000 µM) + Chl (3 000 µM).....	4.4	1.3	21-30
PBA (500 µM) + Chl (3 000 µM).....	4.4	1.5	18-30
PBA (500 µM) + SADH (1 250 µM).....	0	0	0
PBA (500 µM) + ACPC (200 µM).....	4.0	1.4	25-30
BA (1 000 µM) + Chl (3 000 µM).....	6.0	1.5	26-30

(fig. 1D). Up to 11 inflorescences and 6 bunches of grapes each containing more than 30 berries were produced by eight-month-old plants which had been treated with multiple applications of PBA. Seeds extracted from these bunches recorded 80 p. 100 germination.

DISCUSSION

Seedlings of deciduous fruits have a juvenile phase during which they do not normally form flowers (ZIMMERMAN, 1976), and the long generation times of woody plants are a major obstacle to plant breeding. The physiology of phase-change (BRINK, 1962), the changeover from the juvenile non-flowering form to the flowering form of the adult plant, has been little studied in economically-significant species. The juvenile and adult forms of fruit plants such as *Vitis vinifera* are less distinctive than those of *Hedera helix*, a favoured subject for work on the physiology of phase-change.

The juvenile phase of grapevine seedlings is short-lived by comparison with most woody perennials. Depending on the genotype it comprises the first 9-15 nodes of the seedling shoot and the juvenile morphology is characterised by spiral (2/5) phyllotaxy and absence of tendrils (BUGNON & BESSIS, 1968). The onset of the adult condition is denoted by the appearance of the first tendril and by distichous phyllotaxy.

Tendrils of Cabernet Sauvignon seedlings were converted into inflorescences by cytokinin treatment (table 1), a result which is consistent with earlier findings with adult grapevines (SRINIVASAN & MULLINS, 1979). In the present experiments the growth retardant, chlormequat, did not induce flowering when applied alone. However, when chlormequat sprays were followed by, or combined with, PBA treatment, there was a marked increase in flower formation on tendrils as compared with plants which received only PBA (table 2). This finding confirms our earlier work in grape cultivars (SRINIVASAN & MULLINS, 1980) that application of an inhibitor of GA-biosynthesis

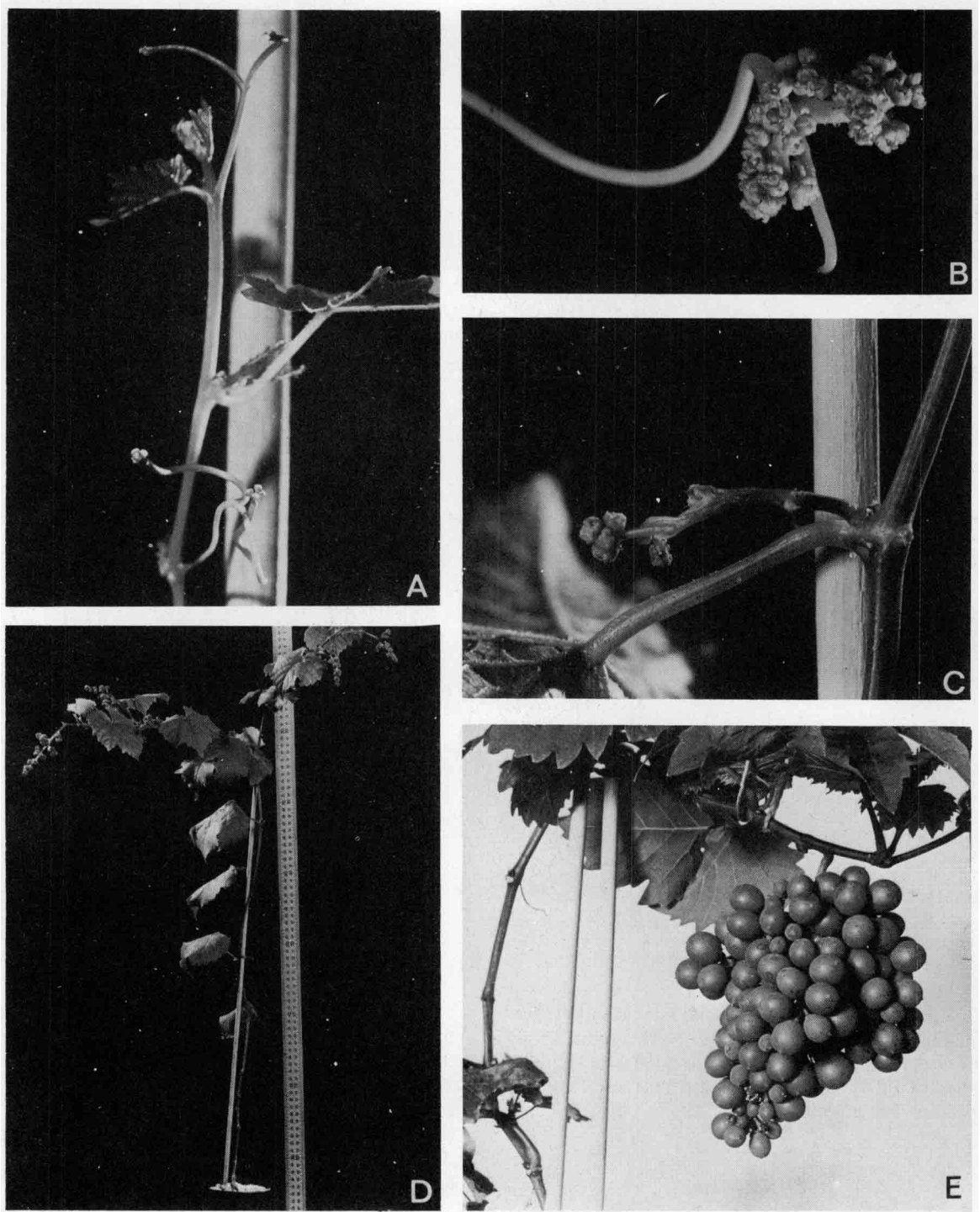


Figure 1

such as chlormequat, followed by cytokinin treatment, is highly effective in transforming potential tendrils into inflorescences, and it supports the view that variation in the endogenous levels of gibberellin and cytokinin is the factor which determines whether the Anlage will grow into a tendril or an inflorescence.

The ease with which tendrils may be converted into inflorescences, and the size of the bunches of grapes which are produced, varies with genotype and the age of the seedling when growth regulators are applied. Muscat Hamburg and Gloryvine are more favourable materials than Cabernet Sauvignon for induction of flowering by growth regulators and the size of inflorescences increases with increasing age of the seedling. In Gloryvine, a male cultivar, it is noteworthy that applications of PBA to tendrils gave rise directly to hermaphrodite flowers. Earlier reports on the feminizing effects of PBA have been concerned with the conversion of pre-formed male flowers into perfect flowers (NEGI & OLMO, 1966). Under some circumstances these effects of PBA on sex expression could limit the usefulness of PBA-induced flowering in plant breeding. Male seedlings, which normally are discarded, would appear as hermaphrodites in PBA-treated populations and their true identity would be hidden.

The conversion of tendrils of very young grapevine

seedlings into inflorescences by growth regulators may have only limited interest for plant breeders because the bunch size, and hence the progeny size, is very small. Moreover, emasculation would be difficult because of the small size of the flowers. Of greater significance is the effect of cytokinin and chlormequat on the precocious formation of inflorescence primordia in the latent buds of 3-month-old seedlings. When induced to grow out by pruning these inflorescences gave rise to bunches with over 200 berries.

The techniques described here need to be refined and extended to a wider range of genotypes but the results obtained so far suggest that use of growth regulators to induce precocious flowering in grapevine seedlings could reduce the breeder's dependence upon natural seasons and permit a significant speeding-up of some aspects of the breeding process.

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Figure 1

- A. Flower formation from the second and the third tendrils of 4-week-old seedlings of Cabernet Sauvignon ($\times 1$).
- B. A tendril in a 6-week-old seedling of Cabernet Sauvignon which has been transformed into an inflorescence. PBA (250 μM) was applied to the shoot tip of the seedling when it was 4-weeks-old ($\times 2$).
- C. A lateral shoot which has produced an inflorescence in place of tendril in a 4-month-old seedling of Muscat Hamburg treated with PBA (500 μM) and chlormequat (3 000 μM) ($\times 1,2$).
- D. Formation of 7 inflorescences in a 6-month-old plant grown from a somatic embryo of Gloryvine (*V. vinifera* \times *V. rupestris*). The inflorescences developed from latent buds following treatment with PBA (500 μM) for three weeks ($\times 0,07$).
- E. A bunch of grapes produced at one metre height by a 7-month-old seedling of Muscat Hamburg. This bunch developed by a tendril of a latent bud activated by pruning. The seedling was sprayed with PBA (500 μM) and chlormequat (3 000 μM) when the seedling was 3 months old ($\times 0,4$).
- A. Formation de fleurs sur les 2^e et 3^e vrilles de plants de semis de Cabernet Sauvignon de 4 semaines ($\times 1$).
- B. Vrille sur un plant de semis de Cabernet Sauvignon de 6 semaines, transformée en inflorescence. Le PBA (250 μM) a été appliqué à l'extrémité de la tige du plant, à 4 semaines ($\times 2$).
- C. Tige latérale qui a produit une inflorescence à la place de vrille chez un plant de semis de Muscat de Hambourg de 4 mois traité au PBA (500 μM) et au chlormequat (3 000 μM) ($\times 1,2$).
- D. Formation de 7 inflorescences chez un plant de 6 mois issu d'un embryon somatique de Gloryvine (*V. vinifera* \times *V. rupestris*). Les inflorescences sont développées dans des bourgeons latents à la suite d'un traitement au PBA (500 μM) pendant 3 semaines ($\times 0,07$).
- E. Une grappe de raisin produite à une hauteur de 1 m par un plant de semis de Muscat de Hambourg de 7 mois. Cette grappe provient de la transformation d'une vrille d'un bourgeon latent forcé par la taille. Le plant de semis avait été traité au PBA (500 μM) et au chlormequat (3 000 μM) quand il avait 3 mois ($\times 0,4$).