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Research Note

Endogenous abscisic acid in juvenile and adult grape (*Vitis vinifera* L. cv. Pinot noir)

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Introduction: After several subcultures, grapevines growing *in vitro* show rejuvenation (Mullins *et al.* 1979). The juvenile characters can be induced by the manipulation of culture conditions. By changing the CO_2 concentration, two distinct morphological patterns were obtained (Fournioux 1995): adult (phyllotaxy 1/2 and presence of tendrils) and juvenile (phyllotaxy 2/5 and lack of tendrils) micropropagated plants.

Some juvenile characteristics may be preserved after acclimatization and transfer into the vineyard: leaves are more jagged, the anthocyanin content is higher and lower fertility is observed in most cultivars (Grenan 1982). As it is difficult to determine the degree of juvenility with morphological characteristics only (Fournioux *et al.* 1998), the use of biochemical markers should be added.

The abscisic acid (ABA) content of a plant depends on age and varies during ontogenesis. ABA acts as an antagonist of gibberellic acid by maintaining the adult stage in *Hedera helix* (Rogler and Hackett 1975). It seems to be one of the factors involved in the control of flowering of *Xanthium strumarium* and as high levels of ABA are supposed to characterize the adult stage, low levels would maintain the juvenile stage (Podolnyi *et al.* 1989).

We have quantified the ABA content of juvenile and adult plants cultivated *in situ* (greenhouse) or *in vitro*, with the aim to investigate the possible relationship between ABA changes and the process of juvenilisation.

Material and Methods: P l a n t m a t e r i a l : Mature cuttings and seeds of *Vitis vinifera* L. cv. Pinot noir were cultivated in a greenhouse (25 °C day, 22 °C night; 16 h light, 80 µmol·m $^{-2}$·s^{-1}). After three months, leaves of seedlings and cuttings were collected from the median part of the shoots. Lateral bud microcuttings produced *in vitro* and obtained after several subcultures from the initial explant of Pinot noir were inserted in 25 x 250 mm culture tubes containing 15 ml of the Galzy medium (Galzy 1969). Cultures were incubated at 28 °C (day) and 24 °C (night) and 16 h light (125 µmol·m $^{-2}$·s^{-1}) at the culture level; fluorescent tubes were used (Grolux, Sylvania, Germany). The adult morphology was obtained by placing cultures in a growth incubator with 1200 µmol·mol $^{-1}$ CO_2 and the juvenile plants were produced under 100 µmol·mol $^{-1}$ CO_2 (Fournioux 1995). Leaves of the median part of the shoots were collected after three months of culture, immediately frozen in liquid nitrogen and lyophilized.

A B A a n a l y s i s : 50 mg of lyophilized leaf tissue were incubated (20 h at 4 °C in the darkness) in 25 ml of 80 % acetone containing the antioxidant butylhydroxytoluene. Known amounts of tritiated ABA (2.22 TBq·mmol, Amersham, UK) were added to estimate losses during processing. After filtration through a Whatman GF/C filter and reduction to aqueous phase by evaporation at 35 °C under vacuum, dialysis was performed as described by Liu and Tillberg (1983). After 20 h at 20 °C in the darkness, the dialysis tube content was collected and the pH was adjusted to 2.5. ABA was extracted against 3 x 30 ml of ethylacetate and the pooled ethylacetate phase evaporated to dryness at 35 °C under vacuum. The residue was dissolved in 3 x 0.5 ml of methanol. 50 µl of the sample were injected into a HPLC (Waters) 4 x 250 mm reverse phase C_{18} column of 5 µm particle size (Lichrospher 100-RP 18 Merck, Germany). ABA was eluted by a methanol:water-5% acetic acid (v:v) solvent gradient (at 0 min: 20 % methanol; from 15 to 20 min: 45 % methanol; from 30 to 35 min: 20 % methanol) at a flow rate of 1 ml·min^{-1}. The ABA fraction (23.5 - 26 min) was collected and ABA was quantified with an indirect competitive ELISA test (Julliard *et al.* 1994).

S t a t i s t i c a l a n a l y s e s : All experiments were replicated 5 times and standard deviation was calculated.

Results and Discussion: The results of the indirect ELISA tests indicate differences in ABA concentrations between juvenile and adult plants, both *in situ* and *in vitro*. As shown in the Table, the leaves of juvenile seedlings cultured *in situ* have significantly lower ABA values than adult leaves. The ABA concentration of *in vitro* material was low in juvenile leaves and high in adult material. There were no significant differences in the ABA content between juvenile leaves from seedlings or from *in vitro* plants. On the other hand, the two categories of adult plants showed a significant difference: the concentration was higher in *in vitro* than in *in situ* plants.

The ABA level may be a biochemical marker of the developmental stage of *Vitis vinifera* L. cv. Pinot noir. This agrees with results of Kataeva *et al.* (1990) with *Hedera*

T a b l e

Endogenous ABA concentrations (ng·g^{-1} dry weight, ± SD) of leaves of grapevines (cv. Pinot noir) cultivated *in situ* and *in vitro*

Juvenile		Adult	
in situ	*in vitro*	*in situ*	*in vitro*
629 ± 34	615 ± 24	926 ± 35	1240 ± 113

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helix, demonstrating that high concentrations were characteristic for the adult stage while small amounts were typical for the juvenile.

The *in vitro* material cultured under 100 μmol·mol $^{-1}$ CO_2 provides a good model to study juvenility because ABA concentration is comparable with that of young greenhouse shoots grown under normal CO_2 atmosphere.

Maturation of Pinot noir, produced *in situ* and *in vitro*, is accompanied by an increase in endogenous ABA. However, in the *in vitro* material the high ABA level seems to be determined not only by its morphological form. The high CO_2 concentration used to obtain this morphology *in vitro* has probably a promoting effect on the ABA concentration in the leaves as well. This could explain the differences between the two culture conditions.

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