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Research Note

# Endogenous polyamine concentrations in juvenile, adult and micropropagated grapevine (*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 are more or less marked according to the culture conditions. By changing $CO_2$ concentration, three distinct morphological patterns were obtained (Fournioux 1995): adult, intermediate (phyllotaxy is 1/2 and none tendril is observed) and juvenile micropropagated plants are produced.

After acclimatization, some juvenile characters may remain: leaves are more jagged, the anthocyanin content is higher and a lower fertility is observed in most cultivars (Grenan 1982). As it is difficult to determine the degree of juvenility with morphologic characters only, the use of biochemical markers should be added.

To our knowledge, only few biochemical studies have been made with grape to understand the transition from the juvenile to the mature phase and, consequently, the process of rejuvenation induced by *in vitro* propagation.

Polyamines participate in the regulation of developmental processes in plants, particularly flowering and the prevention of senescence (Galston and Sawhney 1990). They also seem to play a role in the development of vines cultivated *in vitro* (Martin-Tanguy and Carre 1993). Polyamines are also involved in the juvenility process. Rey *et al.* (1994) showed that the putrescine content and the putrescine/spermidine+spermine ratio were higher in juvenile and micropropagated tissues than in adult tissue of hazel nut.

In the present paper, we report studies on polyamine concentrations in leaves of seedlings, adult shoots and micropropagated plants with the aim to investigate the possible relationship between polyamine changes and the process of juvenility.

**Materials and methods:** Plant material: Hardwood cuttings and seeds of *Vitis vinifera* cv. Pinot noir were cultured in a growth chamber (25 °C day, 22 °C night; 16 h light). After 3 months, leaves of seedlings and adult shoots 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 used. These nodal explants, comprising an axillary bud and a 10 mm section of internode were inserted in 25x250 mm culture tubes containing 20 ml of modified MS medium (Fournioux and Bessis 1993). Cultures were incubated at 28 °C (day) and 24 °C (night) and 16 h light (125 µmol quanta·$m^{-2}$·$s^{-1}$) at the culture level; "Grolux" fluorescent tubes were used (Sylvania, Germany). The adult morphology was obtained by placing cultures in a growth incubator where the $CO_2$ concentration was maintained at 1200 µmol·$mol^{-1}$. The juvenile *in vitro* plants were produced in a low $CO_2$ concentration (100 µmol·$mol^{-1}$). The intermediate morphology was obtained when microcuttings were grown under approximately atmospheric $CO_2$ conditions.

After 3 months of culture, leaves of the median part of shoots of *in vitro*-grown plants were collected of each treatment. Leaf tissues were frozen in liquid nitrogen and lyophilized.

Polyamine analysis: Extraction, purification and dansylation of polyamines were performed as described by Walters and Geuns (1987). Briefly, 60 mg of lyophilized leaves were homogenized in 3 ml of 4 % $HClO_4$ containing 1.7 diaminoheptane as internal standard. After 1 h at 4 °C the homogenate was filtered through glass wool. To 0.2 ml of homogenate, 1 ml of carbonate buffer (pH 9) and 1 ml of dansyl chloride solution (10 mg·$ml^{-1}$ acetone) were added. After heating for 1 h at 60 °C, the dansylated polyamines were extracted with 3 ml of toluene. The extract was loaded on a 0.5 g silica gel column and washed with 5 ml of toluol and 5 ml of toluol-triethylamine (9/1, v/v). The dansylated polyamines were then eluted with 2x3 ml of ethyl acetate and the volume reduced under $N_2$. Polyamines were analyzed by HPLC. The column used was a Lichrosphere 100-RP 18, 25 cm x 4 mm i.d., 5 µm particle size. Samples were eluted from the column with a programmed acetonitrile/water (v/v) solvent gradient (during 2 min: 58 % acetonitrile; after 20 min: 91 % actetonitrile; after 26 min and until 30 min: 58 % acetonitrile; 1.5 ml·$min^{-1}$ flow rate). Dansylated putrescine, diaminopropane, cadaverine, spermidine and spermine (Sigma, St. Louis, USA) were injected as references.

Statistical analysis: All experiments were performed 5 times with 3 replicates per sample. Standard deviations were given in the results.

**Results and Discussion:** *In situ*, spermidine was the most abundant polyamine in adult (65 %) and juvenile (72 %) leaves. This result is in agreement with the observations of Broquedis *et al.* (1989). The amounts of the three detected polyamines were significantly higher in adult than in juvenile leaves. Consequently, total polyamines were higher in adult than in juvenile tissues (Table), but the genotype of seedlings (juvenile status) and cuttings (adult status) were different.

*In vitro*, the spermidine and spermine rates varied at random from one condition to another. Our results showed that the increase of putrescine content was correlated with maturation (Table). The same result was obtained for the putrescine/spermidine+spermine ratio. Rey *et al.* (1994) observed higher putrescine concentrations in juvenile than in adult hazel tissues, which is the opposite of what we ob-

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Table

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

| | Putrescine | Spermidine | Spermine | Ratio Putrescine/ Spermidine+Spermine | Total polyamines |
|---|---|---|---|---|---|
| *in situ* | | | | | |
| juvenile | 51 ± 12 | 853 ± 81 | 276 ± 29 | 0.045 ± 0.009 | 1180 ± 117 |
| adult | 174 ± 12 | 1331 ± 179 | 529 ± 99 | 0.095 ± 0.016 | 2034 ± 281 |
| *in vitro* | | | | | |
| juvenile | 32 ± 5 | 1014 ± 33 | 641 ± 22 | 0.019 ± 0.003 | 1686 ± 47 |
| intermediate | 449 ± 6 | 626 ± 20 | 218 ± 13 | 0.533 ± 0.022 | 1292 ± 27 |
| adult | 1539 ±132 | 1003 ± 35 | 342 ± 30 | 1.143 ± 0.048 | 2884 ± 192 |

served in vine. A comparison between juvenile and adult *in vitro* plants showed that the increase of total polyamines was only due to putrescine. On the other hand, with regard to the intermediate conditions, total polyamines were lower than in the juvenile conditions despite of the putrescine increase. Indeed, both, spermidine and spermine rates, were significantly lower.

Cadaverine has been detected in leaves of Cabernet-Sauvignon (BROQUEDIS *et al.* 1989) and diaminopropane was the major polyamine in *in vitro* plantlets of 41 B (*Vitis vinifera* cv. Chasselas x *Vitis Berlandieri*) (MARTIN-TANGUY *et al.* 1993). However, considering our *in situ* and *in vitro* results, these two polyamines have not been found. According to BROQUEDIS *et al.* (1989), it is possible that different cultivars have different polyamine composition.

The putrescine content and the ratio putrescine/spermidine+spermine were higher in adult tissues than in juvenile tissues. KÖNIGSHOFER (1990) observed a correlation between the rise of putrescine concentration and the maturation process of *Picea abies*.

**Conclusion:** The results of this study have provided evidence that the putrescine content and the putrescine/spermidine+spermine ratio are markers to estimate the degree of juvenility in *Vitis vinifera*, cv. Pinot noir. To confirm the validity of our results, some prospects of research can be proposed. The seedlings used to perform the polyamine analysis described in the present paper were characterized by a 2/5 phyllotaxy and by a lack of tendrils. It would be interesting to perform the same analysis with the same material when its adult morphology (phyllotaxy 1/2 and production of tendrils according to a repetitive patterns) is obtained. These new data would indicate if the transition from the juvenile to the adult status is correlated with the rise of putrescine content. Bound polyamines appear to be involved in reproduction-related metabolism (MARTIN-TANGUY 1985). However, the role of these compounds is poorly understood. Consequently, we also suggest to study their possible effect in the processes of phase-change and ontogenetic maturation.

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