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Research Note

Changes of the polyphenol composition along a cane of a *Vitis vinifera* × *labrusca* seedling

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K e y w o r d s : polyphenol, seedling.

Introduction: Juvenile, adult vegetative, and reproductive phases are the three ontogenetic stages of flowering plants. Besides maturity of woody perennials, which is of great interest for breeders to select elite individuals, juvenility or rejuvenation is desirable for propagation. Meanwhile, morphological, biochemical and molecular markers of juvenility and maturation have been selected for many plant species.

Phytohormones and polyphenols have been proposed as biochemical markers, some of the polyphenol markers being qualitative. Condensed tannins can be used as qualitative markers in chestnut (*Castanea sativa* Mill. and its hybrids) since they were found only in mature material (Fernandez-Lorenzo *et al*. 1999). In walnut hybrids (*Juglans* ssp.), polyphenols, such as hydrojuglone glucoside and myricitrin, accumulated in rejuvenated material via activation of enzymes, e.g. phenylalanine-ammonia-lyase and chalcone synthase (Clardot *et al*. 1992, 1993). In English ivy (*Hedera helix* L.), however, an experimental model plant to study juvenility, the lack of dihydroflavonol reductase activity resulted in a lack of anthocyanins in mature leaf discs (Murray and Hackett 1991). Flavonoids (flavanones, flavones and flavonols) are present only in the aerial parts in the vegetative stage and are detectable in roots in the reproductive stage of *Pyracantha coccinea* only (Fico *et al*. 2000).

Grapevine seedlings need approximately three years from sowing to bloom. Flowering could be induced, however, by treating apices with 6-benzyladenine in 4-week-old seedlings of *Vitis vinifera* L. (Srinivasan and Mullins 1979). This result infers that there is still a great potential to reduce time in grapevine breeding. An impediment to shorten the juvenile period is the lack of knowledge on juvenility in this species. Hence, the objective of this work was to investigate several phenolic compounds along a cane.

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Material and Methods: P l a n t m a t e r i a l : Sixty 2-year-old open-pollinated seedlings of cv. Kyoho (*Vitis vinifera* x *labrusca*) were selected and sampled on September 6. The vines were divided into two groups, 30 vines in each. From each of the 30 vines segments of 5 internodes were cut starting at the basal end. Leaves, axillary buds and bark of 30 segments from vines of the same internode sequence were excised, wrapped with cheese-cloth, immersed and stored in liquid nitrogen.

E x t r a c t i o n a n d a n a l y s i s o f p h e n o l i c c o m p o u n d s : The samples were freeze-dried, ground and sieved to a fine powder. Polyphenols were extracted following Fernandez-Lorenzo *et al.* (1999) with modifications. One hundred mg of sample was pro-eluted with 5 ml petroleum ether (60-90 °C) at room temperature for 36 h and centrifuged at 8,220 g for 10 min. The supernatant was removed. The residue was washed with 5 ml petroleum ether and centrifuged again at 8,220 g for 10 min. After petroleum ether was evaporated in the dark at 30 °C, the residue was extracted with 80 % acetone (2 ml) and exposed to an ultrasound bath for 30 min, extracted overnight at 4 °C, and centrifuged at 12,800 g for 10 min. Five hundred µl of supernatant were collected and evaporated to dryness in a speed-vac. The residue was redissolved in 500 µl of methanol and centrifuged at 18,500 g for 10 min. Four hundred µl of supernatant were collected and evaporated to dryness again. The residue was redissolved in 400 µl HPLC mobile phase, filtered through disposable 0.45 µm nitrocellulose membranes and stored at -35 °C. The extract was diluted 1:10 with HPLC mobile phase before analysis.

The diluted extracts (10 µl) were separated by HPLC (Waters 510) using a reverse phase column (Luna C18, 250 x 4.6 mm, 5 µm). Column temperature was 25 °C. The mobile phase was methanol, glacial acetic acid, and redistilled water (45:3:52, v:v:v); flow rate: 1 ml·min^{-1}. The compounds were detected by their absorbance at 280 nm with a Waters 2487 Dual λ absorbance detector.

Data were collected and processed by N2000 software (Zhejiang University). Phenolic compounds were identified by their retention time comparing them with external standards: (+)-catechin (C-1251), Quercitrin (Q-3001), p-hydroxybenzoic acid (H-5415), rutin (R-5143), phloridzin (P-3449) and chlorogenic acid (C-3878) (Sigma Chemical, St. Louis, MO, USA). Endogenous polyphenols were quantified by the linear regression between peak area and concentration of external standards.

Results and Discussion: Phloridzin was not detectable in all the samples. We found, however, significant amounts of rutin varying irregularly between replicates. The concentrations of catechin, quercitrin, hydroxybenzoic acid and chlorogenic acid in buds, leaves, and bark varied significantly among nodes but negligibly between replicates. Thus data of these 4 polyphenols obtained from two replicates were averaged (Figure).

The amount of catechin was higher in buds than in leaves and bark. There was a general tendency of catechin to increase in bud and bark samples from basal nodes to

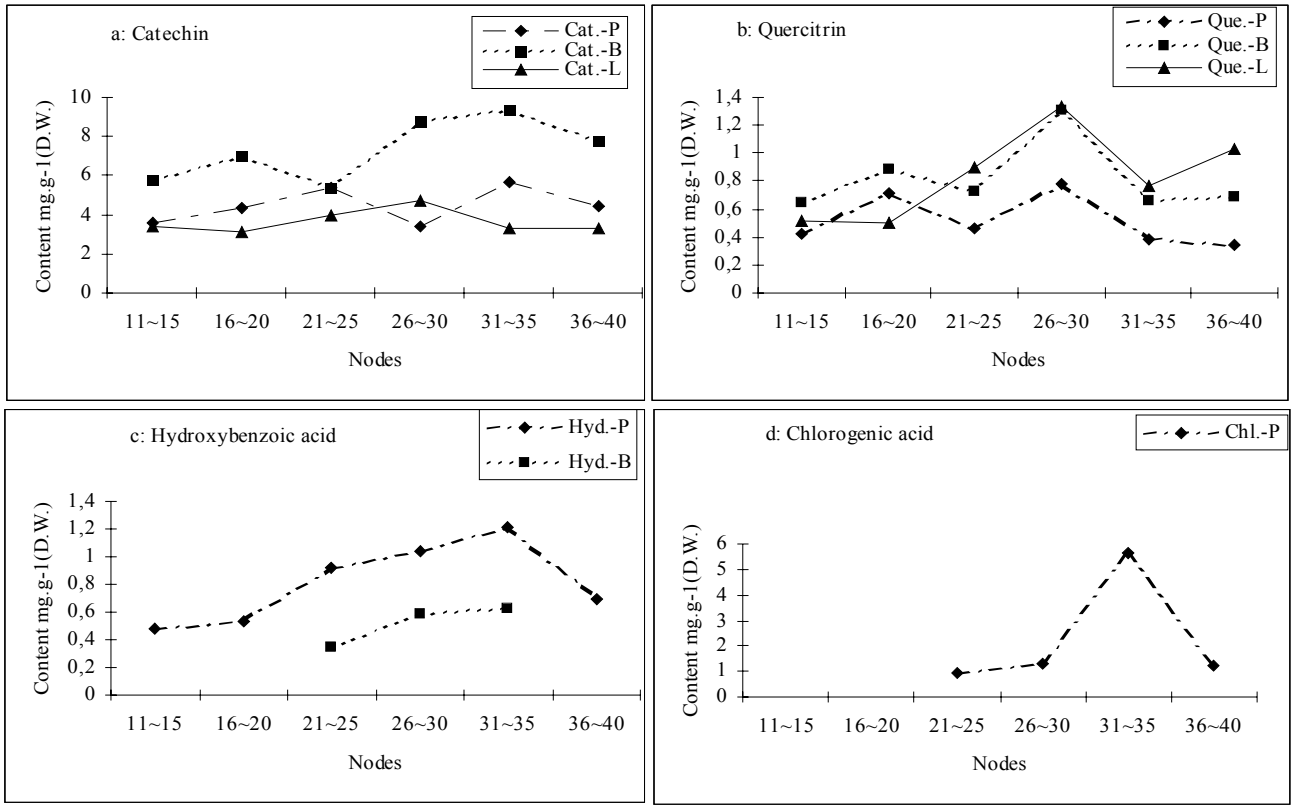

Figure: Dynamics of polyphenols in seedling grapevines. Cat. = catechin, Que. = quercitrin, Hyd. = hydroxybenzoic acid, Chl. = chlorogenic acid, P = phloem, B = buds, L = leaves.

node no. 31-35, but it transiently decreased at node no. 21-25 and no. 26-30 in buds and bark respectively. The leaf catechin level was relatively low.

Quercitrin in buds and bark peaked at the node no.16-20 and no. 26-30 while the highest quercitrin content in leaves was at node no. 26-30.

Chlorogenic acid was not present in leaves and buds of any segment, nor in bark below node no. 20. This phenolic acid appeared only in bark from node no. 21-25 to the apex, the concentration increased gradually from node no. 21-25 to no. 31-35 and dropped at no. 36-40. ZHUO *et al.* (1995) have reported that the first inflorescence was formed at node no. 21of Kyoho seedlings. This was reconfirmed by our investigation (data not presented), indicating that the reproductive stage started at node no. 21. Chlorogenic acid accumulated only in the bark of mature shoot sections.

Hydroxybenzoic acid was not detected in leaf samples, but it occurred in bark; the amount increased gradually from node no. 11-15 to no. 31-35 and declined at node no. 36-40. In bud samples, hydroxybenzoic acid was not detectable until node no. 21-25 and its amount increased promptly in the upper segments of vines.

Both qualitative and quantitative markers are available but qualitative markers are more valuable than quantitative, which can be influenced by genetic, seasonal, and/or environmental factors. According to our findings, changes of chlorogenic acid and hydroxybenzoic acid along a cane were qualitative.

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