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Research Note

Effect of cluster thinning on catechins in berries of *Vitis vinifera* cv. Cabernet Sauvignon

X. J. Zhao[1), 2)], X. Wang[3)], B. Liu[1)], J. Li[4)] , Y. X. Sun[2)], and H. R. Shu[1*)]

[1)] Department of Horticulture, Shandong Agricultural University, Tai'an, China
[2)] Shandong Vine and Wine-making Institute, Jinan, China
[3)] Shandong Analysis and Test Center, Jinan, China
[4)] College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan, China

K e y w o r d s : Cluster thinning, grape, catechin, epicatechin, epigallocatechin.

Introduction: Catechins, namely catechin, epicatechin and epigallocatechin, are important phenolic compounds in the grape berry (Souquet *et al*. 1996, Harbertson *et al.* 2002). These compounds and the tannins are responsible for some major grape and wine organoleptic properties and affect human health (Harbertson *et al*. 2002, Goldberg *et al.* 1998). Cluster thinning has been used in grape production to control crop load. Several studies have examined the effects of cluster thinning on vine growth and berry composition, e.g. soluble solids, titratable acidity, polyphenols, anthocyanin, total nitrogen (Guidoni *et al.* 2002, Palliotti and Cartechini 2000, Morinaga *et al.* 2000), but there is little information on the effect of cluster thinning on catechins in berries. In this paper, we studied the effects of cluster thinning on the catechin contents in berries at different developmental stages.

Material and Methods: The experiment was performed in 2003 on *Vitis vinifera* cv. Cabernet Sauvignon planted in a vineyard in Mile, Yunnan, China, at an altitude of 1,467 m. The vineyard was planted in 1998. Vine spacing was 1 m in north-south oriented rows and 2 m between rows. Vines were trained to double curtains with 15 shoots per vine, each shoot had two clusters.

F i v e t r e a t m e n t s w e r e s t u d i e d : (1) basal cluster was kept, distal cluster was removed at anthesis (9 April) (T1); (2) distal cluster was kept, basal cluster was removed at anthesis (T2); (3) basal cluster was kept, distal cluster was removed at veraison (20 June) (T3); (4) distal cluster was kept, basal cluster was removed at veraison (T4); (5) basal cluster and distal cluster were kept, no cluster removal (CK). For each treatment 40 vines were used.

Three weeks after veraison (10 July) and at ripening (28 July), clusters (3 kg) of each treatment with consistent degrees of maturity and size were harvested and stored at -18 °C. Catechins were analyzed according to Amiot *et al.* (1992) and Mayen *et al.* (1997) with some modifications. Berries (20 g) of each treatment were chosen randomly and put into a homogenizer in 200 ml cold ethanol (65 %) containing sodium metabisulphite (0.5 %). They were extracted in an ice bath for 30 min. The homogenate was centrifuged at 5,000 rpm for 15 min at 6 °C, the residue was discarded. Ethanol was removed from the supernatant by evaporation under vacuum at 40 °C and pigments were eliminated by two successive extractions with petroleum ether (150 ml). After addition of ammonium sulphate (20 %) and metaphosphoric acid (2 %) to the aqueous phase, catechins and other phenolic compounds were extracted three times by ethyl acetate (1:1, v:v). The three organic phases were collected, evaporated and dried under vacuum at 35 °C. The residue was re-dissolved in 5 ml of methanol and the methanolic extract was filtered through Gelman Nylon Acrodisc 13 (0.45 µm) and stored at -20 °C before high performance liquid chromatography (HPLC) analysis. The HPLC system consisted of a Waters 660 pump, a Waters 660 controller, a sample injector with a 10 µl loop, and a Waters 996 photodiode array detector. Evaluation and quantification were made on a Millenium^{32} workstation (Waters, USA).

T h e c h r o m a t o g r a p h i c c o n d i t i o n s w e r e a s f o l l o w s : A VP-ODSC_{18} column (250 mm x 4.6 mm, particle size 5 µm) was used at a flow rate of 1 ml·min^{-1}. Peaks were detected by PAD at 280 nm. The mobile phase (A) was methanol containing formic acid (0.1 %) and (B) water containing formic acid (0.1 %). The best separation was obtained using the following gradient elution: At 0 min 15 % and 85 % B, at 12 min 17 % and 83 % B, at 18 min 18 % and 82 % B, at 33 min 20 % and 80 % B, at 38 min 22 % and 78 % B, at 43 min 24 % and 76 % B, at 60 min 25 % and 75 % B. Commercial standards were purchased from Sigma Chemicals, their purity were all above 98 %. All the calculations concerning the quantitative analysis were performed with external standardization by measuring peak areas (Figure).

Results and Discussion: The concentration of catechins increased during the first stage of berry development, then dropped sharply along with berry ripening and finally tended to be steady (Jordao *et al.* 2001). Around anthesis, reproductive growth is limited by competition among different plant sinks for carbohydrate resources (Lioyd 1980). Basal clusters were better developed than distal clusters. Removing distal clusters would be favorable for basal cluster development.

As indicated in the Table, from three weeks after veraison to ripeness, concentrations of catechin, epicatechin and epigallocatechin decreased in berries of all 4 treatments, but the decrease was different between treatments. The total concentration of three catechins decreased in each of 4 treatments (removal of clusters) and was larger than that in CK (no clusters removal), especially when the distal cluster (T1, T3) was removed. This illustrates that cluster thinning promoted berry development.

Three weeks after veraison, the concentration of catechin, epicatechin and epigallocatechin was markedly dif-

Correspondence to: Dr. H. R. Shu, Department of Horticulture, Shandong Agricultural University, Tai'an, 271018, P.R. China. E-mail: hrshu@sdau.edu.cn

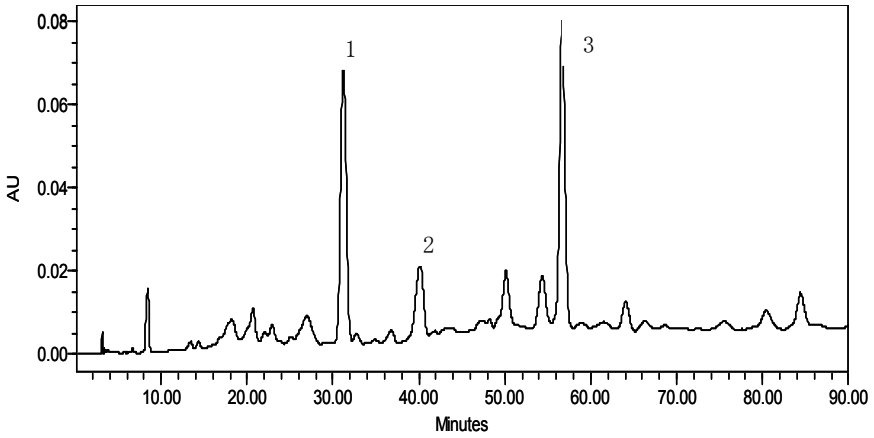

Figure: HPLC profile of (1) catechin, (2) epigallocatechin, (3) epicatechin from grape berries.

T a b l e

Concentrations of catechins ($\mu g \cdot g^{-1}$) in berries at different stages of berry development

	Catechin		Epicatechin		Epigallocatechin		Total catechins		Average berry weight (g)
	A	B	A	B	A	B	A	B	
T1	240 a	108 b	203 a	96 b	235 a	186 a	678 a	287 a	1.04
T2	197 c	107 b	164 b	96 b	204 d	181 b	564 c	180 c	1.04
T3	176 d	84 d	133 e	76 c	195 e	140 d	505 e	206 b	1.06
T4	199 b	114 a	156 c	107 a	218 c	185 a	574 b	167 d	1.03
CK	161 e	100 c	152 d	94 b	221 b	175 c	534 d	164 e	1.03

Note: A: three weeks after veraison; B: at ripening; T1, T2, T3, T4, CK: see Material and Methods. Data with the same letter for each catechins are not significantly different at P = 0.05 as determined by SAS software.

ferent among 5 treatments, respectively. Whether basal or distal inflorescences were removed at anthesis had no significant effect on the concentration of catechin and epicatechin at ripening. For epigallocatechin, there is no difference between the treatment of distal cluster removal at anthesis (T1) and basal cluster removal at veraison (T4), the other 3 treatments being different among each other. It is suggested that the effect of cluster thinning on catechins concentration is complex involving many factors. More work remains to be done to understand the mechanisms.

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