HortScience 24(5):853. 1989.

Estimation of Seed Traces in Grape Berries by Inhibition of Luciferase Activity

M. Perl, U. Lavi, and P. Spiegel Roy
Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel

In examining the adenosine triphosphate (ATP) synthesizing system in seeds, an inhibitory effect of seeds on luciferase activity was detected (4). This study describes possible use of the luciferin–luciferase system for a better definition of the amount of seed traces in grape berries. With growing consumer preference for seedless table grapes and raisins, breeding of stenospermocarpic grape cultivars is of great interest. Stenospermocarpic grapes yield considerably larger seedless berries than parthenocarpic ones, but also often possess seedcoats that develop into hard, stony tissue (2). An objective definition of the amount of seed rudiments would be of considerable value in breeding for seedlessness and in assessing environmental or plant growth regulator effects.

Single, ripe peeled grape berries were homogenized and then suspended in deionized water and brought to a volume of 3 ml. Six replications were used for each test and later, for each cultivar and hybrid seedling examined. After centrifugation (12,000 $\times g$ for 10 min), 25 µl of the supernatant was examined for the inhibition of luciferase (EC 1.13.12.7) activity. When the luciferin–luciferase system is used for ATP quantification, the plot of ATP vs. integrated light flux does not exhibit linearity (3); therefore, various concentrations of standard ATP were analyzed for light-emission values in each experiment. ATP concentration was measured in the presence and absence of grape extract by adding 50 µl firefly-tail extract (Sigma FFT, which includes luciferin and luciferase) in a Packard Tri-carb scintillation counter. Results are expressed as amount of ATP measured, or as the percentage of ATP "lost" in the presence of the inhibitor.

The inhibitory effect of grape seed extracts as a function of seed extract concentration is linear on a semi-logarithmic plot (Fig. 1A). Inhibition was abolished by increasing the amount of ATP (Fig. 1B). Addition of luciferin up to 25 µg/assay did not affect the percentage of inhibition.

Dry residues of a 95% ethanol extract of the grape seeds were boiled for 10 min with 0.1 N NaOH, which, however, did not affect the inhibitory activity; in contrast, boiling with 0.1 N HCl did destroy all inhibitory activity, thus indicating that the active material is a complex organic compound. The inhibitory compound could be extracted from the skin, but not from the pulp. Separation on thin-layer chromatography (5 chloroform : 4 ethyl acetate : 1 formic acid) revealed three spots with an inhibitory effect (R_f 15, 65, 90). These spots reacted with phosphomolybdic acid, as do tannins (5). Commercial tannins (Merck) also inhibited luciferase activity. One grape seed had an inhibitory activity similar to ≈2 mg of commercial tannin.

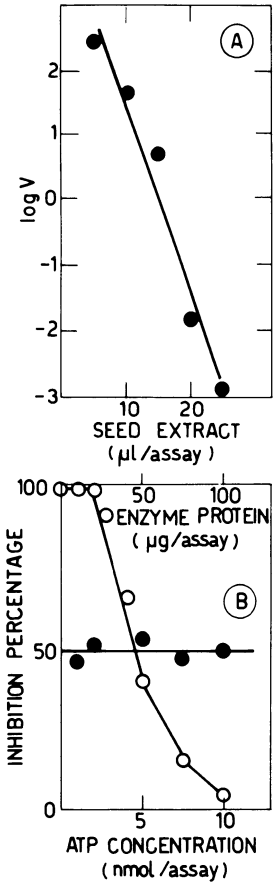

Fig. 1. (**A**) Effect of grape seed extract concentration on luciferase activity. The assay contained 40 µg luciferase protein, 25 µg luciferin, and 15 nmol ATP. V = Rate of luciferase activity expressed as nanomoles of ATP generated per second. The equation line was defined by regression analysis: log V = 1.87 − 0.123 µl seed extract. Correlation coefficient = −0.978 ($P < 0.01$). (**B**) Effect of luciferase (●) (10 nmol ATP, 25 µg luciferin) and ATP (○) (40 µg luciferase, 25 µg luciferin). Concentrations on the inhibitory effect of 20 µl of grape seed extracts. Correlation coefficient between inhibition percentage and concentration of ATP = 0.056 (not significantly different from zero). Inhibition percentage = 100 exp (−0.0314 µg enzyme^2), $P < 0.01$.

The ability of the system to detect seeds and seed traces in three *Vitis vinifera* cultivars and 18 *V. vinifera* hybrids derived from crosses between seeded genotypes (female) and seedless genotypes (pollen parent) was determined (Table 1). A five-member panel also rated fruit of cultivars and hybrids as: 1 = seedless, 2 = seed traces, and 3 = normally developed seeds, according to Olmo and Baris (1). Inhibition of 40% to 50% seems to distinguish between seedless types and those with seed traces.

The proposed method offers a comparatively reliable and accurate determination. Sensory determinations may be biased and weighing seed traces rather inconvenient.

Literature Cited

1. Olmo, H.P. and C. Baris. 1973. Obtention de raisins de table apyrenes. Symp. Intl. sur les Raisins de Table, Limassol, Cyprus.
2. Pearson, H.M. 1932. Parthenocarpy and seed abortion in Vitis vinifera. Proc. Amer. Soc. Hort. Sci. 29:169–175.
3. Perl, M. 1980. An ATP synthesizing system in seeds. Planta 149:1–6.
4. Perl, M. 1981. Reduced nicotinamide-adenine-dinucleotide-phosphorylase: A novel enzyme in seeds. Phytochemistry 20:1791–1793.
5. Stahl, E. and P.J. Schorn. 1965. Hydrophilic constituents of plants, p. 371–391. In: E. Stahl (ed.). Thin-layer chromatography. Springer-Verlag, Berlin.

Table 1. Estimation of the presence of seeds and seed traces by sensory evaluation and by the luciferase-inhibition system.

Cultivar	Plant no. and sensory evaluation[z]	Luciferase inhibition (% ± SD)[y]
Ruby Seedless	1 SL	20 ± 4
	2 SL	10 ± 10
Italia	1 SN	100 ± 0
Muscat Hamburg	1 SN	100 ± 0
Queen Vineyards x Perlette	1 ST	29 ± 22
	2 ST	38 ± 25
	3 ST	0 ± 0
	4 ST	78 ± 14
	5 ST	63 ± 40
Cardinal x Perlette	1 ST	97 ± 8
	2 ST	100 ± 0
	3 ST	100 ± 0
	4 ST	85 ± 16
	5 ST	100 ± 0
	6 ST	81 ± 19
	7 ST	56 ± 39
	8 ST	67 ± 28
	9 ST	81 ± 32
	10 ST	96 ± 5
Queen Vineyards x Flame Seedless	1 SL	18 ± 7
	2 ST	48 ± 45
Dabouki x Perlette	1 ST	100 ± 0

[z]SL = seedless; SN = seeded, ST = seed traces.
[y]Normally seeded grapes = 100% inhibition.

Received for publication 12 Aug. 1988. The technical assistance of Esther Shitrit and I. Baron is appreciated. This research was supported by grant no. 338-80 from the United States–Israel Binational Agricultural Research and Development Fund (BARD). This paper is contribution no. 2441-E, 1988 series, from the ARO, Bet Dagan, Israel. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.