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# Grape Skin and Seed Proanthocyanidins from Monastrell × Syrah Grapes

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In this study, the grape skin and seed proanthocyanidin profiles from Monastrell × Syrah grape (*Vitis vinifera* L.) crosses were determined. Concentration and compositional information in extracts was determined by reversed-phase HPLC after acid-catalyzed cleavage in the presence of excess phloroglucinol. In general, the proanthocyanidin compositions of crosses were qualitatively similar to those of Monastrell and Syrah, but, quantitatively, differences were observed. Consistent with transgressive segregation, the proanthocyanidin concentration in some crosses exceeded that in either parent. To the best of the authors' knowledge, this is the first study to provide information on the inheritance of proanthocyanidin features from *V. vinifera* cultivars. The overall objective of this study is to develop new varieties that are well-adapted to our agro-ecological conditions, as Monastrell is, and with a proanthocyanidin profile that will result in high-quality wines.

**KEYWORDS: Tannin; hybrid; grape; phloroglucinolysis**

## INTRODUCTION

The phenolic composition of grapes at maturity is of great importance to winemakers. Phenolic compounds are important for the quality of red wines in particular, because they contribute bitterness, astringency, and color. Among these phenolics are the proanthocyanidins or condensed tannins, commonly called "tannins" by winemakers, which are oligomeric and polymeric flavan-3-ols linked by C(4)−C(6) or C(4)−C(8) interflavanoid bonds.

Proanthocyanidins are located in the seed and skin of the berry, and their composition and concentration vary depending upon the tissue of origin (*1*, *2*). Seed proanthocyanidins contain a higher proportion of galloylated procyanidins (*3*, *4*), whereas those from skin contain prodelphinidins (*3*, *5*). In addition, skin proanthocyanidins have been shown to have a higher mean degree of polymerization (mDP) than seed proanthocyanidins (*6*, *7*).

Proanthocyanidins and their reaction products have sensory importance in red wines, including color, bitterness, and astringency (*8*). It has been shown, for example, that astringency depends on proanthocyanidin structure such as mDP and the proportion of galloylation (*9*). Because of this, knowledge of the proanthocyanidin structure is important if we are to fully understand their sensory contribution to wine.

The adaptation and selection of vines for different environments in part explain the large observed variation in the qualitative and quantitative flavonoid profile of today's most common winegrape cultivars, and these differences may have technological and nutritional consequences. One way to innovate in viticulture is to develop new varieties; therefore, the objective of this study was to explore the skin and seed proanthocyanidin composition and content of intraspecific hybrids of Monastrell × Syrah, to acquire information for future breeding efforts aimed at the improvement of grape quality through the effects of flavonoids.

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## MATERIALS AND METHODS

A collection of plants arising from crosses between Monastrell and Syrah was used in this study. The study was conducted in 2007 within a 1 ha experimental vineyard located in Bullas (Murcia, southeastern Spain) (latitude 38° 06′ 41″ N, longitude 1° 41′ 50″ W). The climate is semiarid Mediterranean, with hot dry summers and mild winters, having an average annual rainfall of 335 mm. On average, the annual temperature of this area is 15.7 °C, and there are 35−45 frost days per year. In addition, the maximum temperature exceeds 30 °C for 74 days, and the annual evapotranspiration is 1200 mm. The soil was a 60 cm deep clay loam (Typic calciorthid). The varieties of *Vitis vinifera* L. studied were Monastrell, Syrah, and their intraspecific hybrids. The parents were grafted onto 110R rootstock and planted in 1997, whereas the seeds for the intraspecific hybrids were planted in 2000. The training system was a bilateral cordon trellised to a three-wire vertical system with drip irrigation capabilities. Planting density was 2.5 m between rows and 1.25 m between vines. Vines were pruned to two two-bud spurs (four nodes).

Three microsatellite markers were used to detect and exclude plants derived from Monastrell self-pollination and plants derived from pollen donors other than Syrah. The three microsatellite markers of < ab × cd > type were (allelic size, in base pairs, indicated in parentheses): VMC16D4 ( < 154/170 × 168/208 > ),

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VVIP31 (<178/190 × 181/188>), and VVIM93 (<115/122 × 108/126>), according to Ruiz-García et al. (unpublished data). Total DNA was extracted from approximately 20 mg of young frozen leaves using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Genotyping was carried out as described in Adam-Blondon et al. (*10*). PCR products were separated by capillary electrophoresis performed on an ABI Prism 3100 genetic analyzer, and the fragments were sized using GeneMapper software (Applied Biosystems, Carlsbad, CA). PCR products were separated by capillary electrophoresis performed on an ABI Prism 3100 genetic analyzer (Applied Biosystems).

Monastrell and Syrah grape samples were sourced from the same and different vineyards to provide a representative sample. Grapes were harvested at a total soluble solids content between 23 and 27 °Brix. All samplings were made in triplicate. Grape samples were kept frozen (−20 °C) until extraction and analysis.

**Proanthocyanidin Extraction and Analysis.** The seeds and skins of 10 berries were separated from the mesocarp and rinsed with distilled−deionized water. Whole seeds and skins, previously ground to a powder with liquid nitrogen, were extracted separately in covered Erlenmeyer flasks with 10 mL of 2:1 acetone/water at room temperature for 24 h on an orbital shaker at 200 rpm. To minimize proanthocyanidin oxidation, solutions were sparged with nitrogen and the extraction was carried out in the dark. Following extraction, the extract was concentrated under reduced pressure at 35 °C to remove acetone and then lyophilized to a dry powder. This powder was redissolved in 2 mL of methanol in a volumetric flask.

Skin and seed proanthocyanidins were determined according to the method described by Kennedy and Jones (*11*), under modified HPLC conditions (*12*), as follows. A solution of 0.2 N HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid, was prepared (phloroglucinolysis reagent). The methanolic extract was reacted with the phloroglucinolysis reagent (1:1) in a water bath for 20 min at 50 °C and then combined with 5 volumes of 40 mM aqueous sodium acetate to stop the reaction.

The reversed-phase HPLC method used to analyze the phloroglucinol adducts consisted of two 100 mm × 4.6 mm i.d., 5 μm, Chromolith RP-18e columns connected in series and protected by a 4 mm × 4 mm i.d., 5 μm, guard column composed of the same material, all purchased from EM Science (Gibbstown, NJ). The method utilized a binary gradient with water containing 1% v/v aqueous acetic acid (mobile phase A) and acetonitrile containing 1% v/v acetic acid (mobile phase B). Eluting peaks were monitored at 280 nm, and the elution conditions were as follows: column temperature, 30 °C; 3.0 mL/min; 3% B for 4 min, a linear gradient from 3 to 18% B in 10 min, and 80% B for 2 min. The column was washed with 3% B for 2 min before the next injection.

Proanthocyanidin cleavage products were estimated using their response factors relative to (+)-catechin, which was used as the quantitative standard. To calculate the apparent mDP, the sum of all subunits (flavan-3-ol monomer and phloroglucinol adducts, in moles) was divided by the sum of all flavan-3-ol monomers (in moles).

## RESULTS AND DISCUSSION

**Proanthocyanidin Profiles of Syrah and Monastrell Parents.** The concentration of skin and seed proanthocyanidins (mean, minimum, and maximum) for Syrah, Monastrell, and their red and white grape-bearing hybrids are shown in **Tables 1** and **2**. In addition, the proanthocyanidin compositions for the parents and hybrids are shown in **Tables 3** and **4**.

Seeds from Syrah had a higher seed proanthocyanidin quantity (2.28 mg/berry) than skin (0.39 mg/berry). In other studies on Syrah, Harbertson et al. (*13*) reported 1.4 mg/berry for seed and 0.5 mg/berry for skin, whereas Ristic et al. (*14*) reported 1.61−1.93 mg/berry for seed and 0.92−1.15 mg/berry for skin. The observed variation may be due to differences in analytical method in the case of Harbertson et al. (*13*), although site, vintage, and source of fruit have been observed to have an effect in other studies (*15*−*17*).

**Table 1.** Concentrations of Skin Proanthocyanidins from Monastrell, Syrah, and Their Intraspecific Hybrids

| | mg/kg | μg/berry | μg/g |
|---|---|---|---|
| | Red Hybrids | | |
| Syrah | 289 | 392 | 2318 |
| Monastrell | 440 | 706 | 2881 |
| MxS 0 | 608 | 1379 | 5969 |
| Mx S 4 | 1284 | 1866 | 10871 |
| MxS 8 | 1185 | 1677 | 10117 |
| MxS 26 | 550 | 965 | 5095 |
| MxS 28 | 1201 | 1374 | 7319 |
| MxS 31 | 844 | 1208 | 7285 |
| MxS 34 | 456 | 592 | 3387 |
| MxS 38 | 824 | 858 | 4219 |
| MxS 3 | 1078 | 1362 | 6195 |
| MxS 11 | 480 | 822 | 3532 |
| MxS 20 | 141 | 204 | 1074 |
| MxS 21 | 466 | 660 | 3747 |
| MxS 27 | 405 | 632 | 3258 |
| MxS 37 | 807 | 1601 | 6832 |
| MxS 42 | 804 | 1429 | 6943 |
| MxS 46 | 433 | 487 | 2607 |
| MxS 47 | 1065 | 1016 | 9447 |
| MxS 56 | 800 | 1228 | 6777 |
| MxS 57 | 500 | 857 | 3861 |
| MxS 62 | 1118 | 1321 | 8929 |
| MxS 66 | 705 | 684 | 5132 |
| MxS 71 | 483 | 926 | 3829 |
| MxS 75 | 810 | 1301 | 6245 |
| MxS 76 | 334 | 515 | 2381 |
| MxS 97 | 551 | 848 | 4938 |
| MxS 114 | 298 | 580 | 2410 |
| MxS 117 | 385 | 433 | 2476 |
| mean | 689 | 993 | 5366 |
| minimum | 141 | 204 | 1074 |
| maximum | 1248 | 1866 | 10871 |
| | White Hybrids | | |
| MxS 1 | 515 | 747 | 4123 |
| MxS 9 | 493 | 1132 | 4579 |
| MxS 14 | 465 | 791 | 4621 |
| MxS 15 | 1005 | 1386 | 8055 |
| MxS 19 | 711 | 1263 | 6594 |
| MxS 30 | 369 | 576 | 2940 |
| MxS 40 | 272 | 315 | 2044 |
| MxS 59 | 454 | 872 | 4816 |
| MxS 65 | 653 | 804 | 5973 |
| MxS 73 | 758 | 967 | 5906 |
| MxS 82 | 494 | 720 | 6114 |
| MxS 91 | 363 | 402 | 3509 |
| MxS 94 | 876 | 1007 | 7459 |
| MxS 100 | 397 | 573 | 2847 |
| MxS 118 | 844 | 1037 | 7366 |
| mean | 578 | 839 | 5130 |
| minimum | 272 | 315 | 2044 |
| maximum | 1005 | 1386 | 8055 |

With regard to proanthocyanidin composition (**Tables 3** and **4**) the results can be compared with those obtained by other authors in the same or different varieties using the phloroglucinol method for proanthocyanidin determination. The main constituent of skin terminal subunits in Syrah was catechin, which represented 77.7 mol %, similar to Downey et al. (*18*), and no (−)-epicatechin-3-*O*-gallate was detected, similar to Hanlin and Downey (*15*).

**Table 2.** Concentration of Seed Proanthocyanidins from Monastrell, Syrah, and Their Intraspecific Hybrids

| | mg/kg | μg/berry | μg/g |
|---|---|---|---|
| | | Red Hybrids | |
| Syrah | 1676 | 2278 | 46040 |
| Monastrell | 1998 | 3205 | 29218 |
| MxS 0 | 1042 | 2365 | 24490 |
| Mx S 4 | 1978 | 2874 | 43951 |
| MxS 8 | 2705 | 3829 | 41557 |
| MxS 26 | 2785 | 4886 | 40873 |
| MxS 28 | 2839 | 3247 | 65709 |
| MxS 31 | 2908 | 4166 | 33701 |
| MxS 34 | 2197 | 2852 | 47873 |
| MxS 38 | 3613 | 3758 | 60313 |
| MxS 3 | 2634 | 3329 | 32127 |
| MxS 11 | 2701 | 4628 | 31335 |
| MxS 20 | 2158 | 3111 | 37951 |
| MxS 21 | 2214 | 3135 | 49088 |
| MxS 27 | 1944 | 3032 | 40831 |
| MxS 37 | 1890 | 3750 | 27672 |
| MxS 42 | 1925 | 3423 | 24095 |
| MxS 46 | 1329 | 1496 | 25907 |
| MxS 47 | 5172 | 4938 | 84510 |
| MxS 56 | 2125 | 3264 | 39429 |
| MxS 57 | 2720 | 4661 | 38532 |
| MxS 62 | 2986 | 3526 | 48505 |
| MxS 66 | 1739 | 1688 | 35711 |
| MxS 71 | 1733 | 3324 | 40033 |
| MxS 75 | 2680 | 4307 | 38948 |
| MxS 76 | 1305 | 2008 | 35845 |
| MxS 97 | 2154 | 3312 | 26115 |
| MxS 114 | 1801 | 3509 | 30271 |
| MxS 117 | 3413 | 3838 | 48547 |
| mean | 2395 | 3417 | 40501 |
| minimum | 1042 | 1496 | 24095 |
| maximum | 5172 | 4938 | 84510 |
| | | White Hybrids | |
| MxS 1 | 2293 | 3323 | 42302 |
| MxS 9 | 574 | 1319 | 15602 |
| MxS 14 | 1679 | 2856 | 26099 |
| MxS 15 | 3116 | 4297 | 41773 |
| MxS 19 | 148 | 263 | 46225 |
| MxS 30 | 966 | 1509 | 28002 |
| MxS 40 | 2684 | 3109 | 37485 |
| MxS 59 | 956 | 1835 | 21490 |
| MxS 65 | 2855 | 3515 | 42800 |
| MxS 73 | 2301 | 2936 | 46754 |
| MxS 82 | 1438 | 2100 | 31895 |
| MxS 91 | 1511 | 1671 | 33569 |
| MxS 94 | 1206 | 1387 | 31031 |
| MxS 100 | 1511 | 2179 | 34569 |
| MxS 118 | 1839 | 2260 | 37383 |
| mean | 1672 | 2304 | 34465 |
| minimum | 148 | 263 | 15602 |
| maximum | 3116 | 4297 | 46754 |

With regard to skin proanthocyanidin extension subunits, (−)-epicatechin and (−)-epigallocatechin dominated (65 and a 24.8 mol %, respectively), whereas the percentages of catechin and (−)-epicatechin-3-*O*-gallate were very low, consistent with others (*11*, *18*). When the results of Syrah were compared with those of Cabernet Sauvignon and Merlot, some compositional results were similar (*15*, *17*, *19*−*21*), although it is very important to point to the large differences found by some authors, within the same study, depending on the year (*15*, *17*). These differences are much larger than those reported among different clones, such is the case in Carmenere (*22*).

**Table 3.** Structural Characteristics of Skin Proanthocyanidins from Monastrell, Syrah, and Their Intraspecific Hybrids[a]

| | mDP | %terC | %terEC | %extC | %extEC | %extEGC | %extECG |
|---|---|---|---|---|---|---|---|
| Syrah | 13.9 | 77.8 | 22.2 | 3.1 | 65.1 | 24.8 | 7.0 |
| Monastrell | 27.5 | 78.6 | 21.4 | 1.7 | 65.7 | 29.8 | 2.7 |
| | | | | Red Hybrids | | | |
| MxS 0 | 22.4 | 82.0 | 18.0 | 2.2 | 48.9 | 47.4 | 1.5 |
| Mx S 4 | 23.0 | 81.8 | 18.3 | 2.6 | 50.6 | 45.0 | 1.8 |
| MxS 8 | 27.0 | 72.8 | 27.2 | 1.8 | 55.0 | 40.8 | 2.5 |
| MxS 26 | 26.6 | 79.5 | 20.5 | 1.5 | 54.6 | 42.0 | 1.9 |
| MxS 28 | 40.5 | 81.1 | 18.9 | 1.4 | 50.4 | 46.5 | 1.8 |
| MxS 31 | 16.1 | 78.9 | 21.1 | 2.6 | 66.1 | 28.4 | 2.9 |
| MxS 34 | 36.4 | 85.3 | 14.7 | 1.5 | 55.5 | 39.0 | 4.1 |
| MxS 38 | 31.4 | 72.2 | 27.8 | 1.2 | 50.3 | 45.4 | 3.2 |
| MxS 3 | 30.7 | 86.5 | 13.5 | 1.7 | 52.5 | 43.1 | 2.7 |
| MxS 11 | 20.3 | 71.5 | 28.5 | 2.0 | 67.3 | 27.9 | 2.8 |
| MxS 20 | 12.8 | 76.7 | 23.3 | 7.3 | 54.6 | 37.4 | 0.8 |
| MxS 21 | 23.3 | 58.2 | 41.8 | 2.4 | 57.4 | 38.1 | 2.1 |
| MxS 27 | 24.7 | 77.9 | 22.1 | 2.4 | 61.7 | 33.8 | 2.2 |
| MxS 37 | 24.3 | 84.4 | 15.6 | 1.7 | 55.7 | 39.1 | 3.6 |
| MxS 42 | 29.9 | 83.1 | 16.9 | 1.8 | 48.8 | 47.7 | 1.6 |
| MxS 46 | 11.9 | 87.4 | 12.6 | 2.7 | 61.4 | 31.5 | 4.4 |
| MxS 47 | 26.0 | 85.8 | 14.2 | 1.6 | 54.8 | 38.9 | 4.6 |
| MxS 56 | 20.6 | 82.4 | 17.6 | 2.4 | 52.4 | 43.3 | 1.9 |
| MxS 57 | 13.9 | 88.0 | 12.0 | 3.2 | 63.6 | 30.0 | 3.3 |
| MxS 62 | 36.7 | 79.0 | 21.0 | 1.9 | 41.2 | 55.0 | 1.9 |
| MxS 66 | 19.2 | 65.6 | 34.4 | 1.5 | 60.5 | 35.8 | 2.2 |
| MxS 71 | 16.5 | 76.5 | 23.5 | 3.0 | 58.9 | 34.4 | 3.7 |
| MxS 75 | 56.4 | 67.6 | 32.4 | 1.2 | 40.0 | 56.2 | 2.6 |
| MxS 76 | 34.1 | 78.6 | 21.5 | 2.6 | 48.2 | 46.8 | 2.4 |
| MxS 97 | 29.3 | 76.2 | 23.8 | 1.4 | 44.0 | 49.6 | 5.1 |
| MxS 114 | 20.8 | 80.7 | 19.3 | 1.5 | 48.3 | 46.0 | 4.2 |
| MxS 117 | 21.4 | 85.1 | 14.9 | 1.5 | 72.4 | 24.4 | 1.8 |
| mean | 25.7 | 78.7 | 21.3 | 2.1 | 54.6 | 40.5 | 2.7 |
| minimum | 11.9 | 58.2 | 12.0 | 1.2 | 40.0 | 24.4 | 2.7 |
| maximum | 56.4 | 88.0 | 41.8 | 7.3 | 72.4 | 56.2 | 5.1 |
| | | | | White Hybrids | | | |
| MxS 1 | 32.9 | 89.0 | 11.0 | 2.1 | 49.8 | 46.9 | 2.4 |
| MxS 9 | 18.8 | 95.7 | 4.3 | 3.6 | 63.1 | 31.7 | 4.2 |
| MxS 14 | 26.4 | 90.8 | 9.2 | 2.7 | 68.5 | 27.6 | 3.9 |
| MxS 15 | 21.3 | 92.0 | 8.0 | 2.2 | 60.6 | 36.0 | 3.0 |
| MxS 19 | 32.6 | 86.0 | 14.0 | 1.8 | 54.8 | 42.1 | 3.1 |
| MxS 30 | 29.6 | 88.8 | 11.2 | 1.3 | 55.3 | 42.8 | 1.5 |
| MxS 40 | 22.9 | 100.0 | 0.0 | 2.4 | 66.2 | 30.5 | 2.6 |
| MxS 59 | 30.0 | 90.8 | 9.2 | 1.8 | 49.5 | 47.3 | 2.8 |
| MxS 65 | 18.0 | 93.1 | 7.0 | 1.6 | 67.4 | 30.1 | 2.6 |
| MxS 73 | 31.5 | 90.8 | 9.2 | 1.8 | 56.0 | 41.3 | 2.1 |
| MxS 82 | 30.3 | 90.1 | 9.9 | 1.6 | 48.4 | 48.0 | 3.7 |
| MxS 91 | 35.1 | 100.0 | 0.0 | 1.6 | 66.9 | 30.9 | 1.8 |
| MxS 94 | 29.0 | 90.3 | 9.7 | 1.1 | 57.7 | 40.4 | 1.9 |
| MxS 100 | 37.4 | 100.0 | 0.0 | 2.5 | 61.7 | 35.5 | 1.1 |
| MxS 118 | 33.2 | 86.6 | 13.4 | 1.1 | 46.6 | 51.9 | 0.8 |
| mean | 28.6 | 92.2 | 7.7 | 2.0 | 58.3 | 38.8 | 2.5 |
| minimum | 18.8 | 86.0 | 0.0 | 1.1 | 46.6 | 27.6 | 0.8 |
| maximum | 33.2 | 100.0 | 14.0 | 3.6 | 68.5 | 51.9 | 4.2 |

[a] mDP, mean degree of polymerization; %ter, terminal units; %ext, extension units; C, catechin; EC, epicatechin; EGC, epigallocatechin; ECG, epicatechin gallate.

**Table 4.** Structural Characteristics of Seed Proanthocyanidins from Monastrell, Syrah, and Their Intraspecific Hybrids[a]

| | mDP | %terC | %terEC | %terECG | %extC | %extEC | %extECG |
|---|---|---|---|---|---|---|---|
| Syrah | 3.7 | 38.1 | 51.1 | 10.8 | 8.6 | 77.6 | 13.9 |
| Monastrell | 8.3 | 44.3 | 29.4 | 26.3 | 7.8 | 77.5 | 14.7 |
| | | | | Red Hybrids | | | |
| MxS 0 | 4.3 | 43.4 | 35.2 | 21.4 | 8.1 | 78.4 | 13.5 |
| Mx S 4 | 5.3 | 55.1 | 25.7 | 19.2 | 10.7 | 73.6 | 15.7 |
| MxS 8 | 5.3 | 55.5 | 27.5 | 17.1 | 9.2 | 74.2 | 16.6 |
| MxS 26 | 5.2 | 50.9 | 32.3 | 16.9 | 6.5 | 77.8 | 15.7 |
| MxS 28 | 3.4 | 57.4 | 28.2 | 14.4 | 6.7 | 78.8 | 14.6 |
| MxS 31 | 6.2 | 47.3 | 31.6 | 21.1 | 8.8 | 76.5 | 14.6 |
| MxS 34 | 4.9 | 49.9 | 30.7 | 19.4 | 9.0 | 77.5 | 13.6 |
| MxS 38 | 8.0 | 48.2 | 24.2 | 27.6 | 6.7 | 77.5 | 15.9 |
| MxS 3 | 4.7 | 47.0 | 31.3 | 21.7 | 10.1 | 80.9 | 9.1 |
| MxS 11 | 7.9 | 53.4 | 22.6 | 24.0 | 8.0 | 79.5 | 12.5 |
| MxS 20 | 4.9 | 48.4 | 36.8 | 14.8 | 8.1 | 79.7 | 12.2 |
| MxS 21 | 10.5 | 46.9 | 24.8 | 28.3 | 11.9 | 70.8 | 17.4 |
| MxS 27 | 5.8 | 55.8 | 24.7 | 19.6 | 9.9 | 76.1 | 14.1 |
| MxS 37 | 5.5 | 50.1 | 28.3 | 21.6 | 7.1 | 77.7 | 15.2 |
| MxS 42 | 5.5 | 51.2 | 27.9 | 20.9 | 8.4 | 78.6 | 13.0 |
| MxS 46 | 5.1 | 53.8 | 28.3 | 17.9 | 6.2 | 78.4 | 15.4 |
| MxS 47 | 6.3 | 53.6 | 21.0 | 25.4 | 8.4 | 72.2 | 19.4 |
| MxS 56 | 8.8 | 41.8 | 25.4 | 32.8 | 8.2 | 73.8 | 18.0 |
| MxS 57 | 5.2 | 66.9 | 17.3 | 15.8 | 16.0 | 70.7 | 13.3 |
| MxS 62 | 7.0 | 43.4 | 25.9 | 30.7 | 10.6 | 73.7 | 15.7 |
| MxS 66 | 7.7 | 44.7 | 33.1 | 22.2 | 9.2 | 75.0 | 15.8 |
| MxS 71 | 5.2 | 49.7 | 32.9 | 17.4 | 5.8 | 79.6 | 14.7 |
| MxS 75 | 9.0 | 41.7 | 32.1 | 26.2 | 6.7 | 74.3 | 19.0 |
| MxS 76 | 6.6 | 35.1 | 41.4 | 23.5 | 5.7 | 78.6 | 15.7 |
| MxS 97 | 8.3 | 53.2 | 20.5 | 26.3 | 12.1 | 75.6 | 12.3 |
| MxS 114 | 3.7 | 50.5 | 34.9 | 14.6 | 11.0 | 76.9 | 12.1 |
| MxS 117 | 3.8 | 49.8 | 34.8 | 15.4 | 10.7 | 76.8 | 12.5 |
| mean | 6.1 | 49.8 | 28.9 | 21.3 | 8.9 | 76.4 | 14.7 |
| minimum | 3.4 | 35.1 | 17.3 | 14.4 | 5.7 | 70.8 | 9.1 |
| maximum | 10.5 | 66.9 | 41.4 | 32.8 | 16.0 | 80.9 | 19.4 |
| | | | | White Hybrids | | | |
| MxS 1 | 5.2 | 46.8 | 32.0 | 21.2 | 7.8 | 78.4 | 13.9 |
| MxS 9 | 3.4 | 66.1 | 25.2 | 8.7 | 15.9 | 73.5 | 10.6 |
| MxS 14 | 4.3 | 37.4 | 43.9 | 18.7 | 7.1 | 81.2 | 11.7 |
| MxS 15 | 4.0 | 31.6 | 51.2 | 17.2 | 8.7 | 80.7 | 10.5 |
| MxS 19 | 3.6 | 55.8 | 34.3 | 9.9 | 13.5 | 72.9 | 13.7 |
| MxS 30 | 4.9 | 44.7 | 35.9 | 19.5 | 8.5 | 78.0 | 13.5 |
| MxS 40 | 6.3 | 60.5 | 19.2 | 20.3 | 15.1 | 69.8 | 15.2 |
| MxS 59 | 5.1 | 38.1 | 46.6 | 15.3 | 11.1 | 78.0 | 11.0 |
| MxS 65 | 4.3 | 39.0 | 35.5 | 25.6 | 7.9 | 79.6 | 12.5 |
| MxS 73 | 4.2 | 51.0 | 23.7 | 25.4 | 7.6 | 77.1 | 15.3 |
| MxS 82 | 6.3 | 47.8 | 32.1 | 20.1 | 10.7 | 74.8 | 14.5 |
| MxS 91 | 8.2 | 62.0 | 22.3 | 15.7 | 8.8 | 80.2 | 11.1 |
| MxS 94 | 6.8 | 39.3 | 36.2 | 24.5 | 4.4 | 82.3 | 13.3 |
| MxS 100 | 6.4 | 47.3 | 31.0 | 21.7 | 9.9 | 74.8 | 15.3 |
| MxS 118 | 5.8 | 48.7 | 33.4 | 18.0 | 6.9 | 84.4 | 8.7 |
| mean | 5.3 | 47.8 | 33.5 | 18.7 | 9.7 | 77.7 | 12.7 |
| minimum | 3.4 | 31.6 | 19.2 | 8.7 | 4.4 | 69.8 | 8.7 |
| maximum | 8.2 | 66.1 | 51.2 | 25.6 | 15.9 | 84.4 | 15.3 |

[a] mDP, mean degree of polymerization; %ter, terminal units; %ext, extension units; C, catechin; EC, epicatechin; EGC, epigallocatechin; ECG, epicatechin gallate.

The major difference between skin and seed proanthocyanidins was the absence of (−)-epigallocatechin and a higher percentage of (−)-epicatechin-3-*O*-gallate in seeds (**Tables 3** and **4**). When the results of Downey et al. (*18*) were used for comparison, higher percentages of terminal (−)-epicatechin and lower percentages of terminal (+)-catechin and (−)-epicatechin-3-*O*-gallate were observed in our study, and with regard to extension subunits, higher (−)-epicatechin and lower (−)-epicatechin-3-*O*-gallate were reported.

Our reported value for the Syrah skin and seed mDP (**Tables 3** and **4**) was generally lower than that reported by others (*11*,*18*,*23*). For example, Cosme et al. (*23*), using the thiolysis method, found an mDP of 5.0 in Syrah seeds and an mDP of 27 in skins, both higher than our reported values.

With regard to Monastrell proanthocyanidin, this is the first time that its profile has been studied. The concentration of Monastrell proanthocyanidins in the skins was higher than that in Syrah, whereas the mDP was twice that of Syrah (**Tables 1**−**4**). The percentages at which each terminal and extension subunit appeared in skin were very similar to those found in Syrah, with slightly higher percentages of (−)-epigallocatechin extension subunits and lower percentages of (−)-epicatechin-3-*O*-gallate extension subunits. With regard to seed proanthocyanidin amount, Monastrell contained lower quantities than Syrah when expressed as micrograms per gram of seed. However, due to the greater seed size, Monastrell contained a higher concentration when expressed as milligrams per berry. The seed extension subunit proanthocyanidin compositions were similar for both Monastrell and Syrah seeds, with only small differences found in terminal subunits.

**Proanthocyanidin Profile of Monastrell × Syrah Hybrids.** Twenty-seven plants bearing red grapes and 15 plants bearing white grapes, both arising from the cross between Monastrell × Syrah, were studied. The presence of plants bearing white grapes indicated the heterozygous nature of both Monastrell and Syrah grapes with regard to the genes controlling anthocyanin synthesis. This makes them very interesting for studying flavonoid profiles.

With regard to skin proanthocyanidins, the mean concentration found in the red grapes was higher than that found in either parent, reaching around 2 times higher than the values found for Syrah grapes. Only one red hybrid showed a lower concentration than Syrah skins. The highest value observed was 3 times higher than that found in Monastrell grapes, and six hybrids contained > 1000 mg/kg of skin proanthocyanidins. For white grapes, the mean proanthocyanidin content was lower than for red hybrids but still higher than the values found in Syrah and Monastrell. The hybrid presenting the lowest value showed quantities very similar to those detected in Syrah. Eleven of the 15 hybrids showed higher values than Monastrell grapes.

With regard to seed proanthocyanidins in red-skinned grapes, when expressed as micrograms per gram of seed, the mean value was slightly lower than for Syrah seeds, although one hybrid presented twice the quantity found in Syrah seeds. When expressed as milligrams per kilogram of fruit, the mean value of red grape seeds was higher than that for both parents. One hybrid had very high concentration (MxS 47). No correlation was found between the proanthocyanidin concentration in seeds and skin (data not shown).

In the seeds of white grapes, when expressed as micrograms per gram, the mean value was slightly lower in Syrah and the maximum value was only slightly higher than Monastrell. When expressed as milligrams per kilogram, the mean value was similar to that for Syrah and lower than for red grape hybrids. One hybrid contained very low proanthocyanidin quantities.

With regard to proanthocyanidin concentration distribution for the hybrids, it can be seen that a substantial number of individuals showed values that did not fall into the range normally associated with Monastrell and Syrah. The appearance of individuals that fall outside the normal range of their parental

phenotypes is called transgressive segregation (*24*), a phenomenon that is particularly attractive as a mechanism for large and rapid changes in a given population. It is frequent in intraspecific crosses and in domesticated populations. Genetic studies indicate that transgressive segregation mostly results from the appearance in individual genotypes of a combination of alleles from both parents that have effects in the same direction (complementary gene action). If the segregation of a given trait is manifested mainly in one direction (as happens in the current study), this would imply that the trait has experienced fairly constant directional selection (*25*).

Some authors have found values as high as the maximum found in the hybrids. Fujita et al. (*26*) reported 4.5 mg/berry in Cabernet Sauvignon seeds and 2.0 mg/berry in skins; Fernández et al. (*22*) found 1.8 mg/berry in Carmenere skins, and Cosme et al. (*23*) found 91.0 mg/g in Cabernet Sauvignon seeds.

In red skins (**Table 3**), the mDP was close to that for Monastrell, although values as high as 56.3 were found. Among the highest values reported in the literature for mDP are 43 in Cabernet Sauvignon and 45 in Syrah (*23*) and 42 in Pinot noir (*27*). In skin proanthocyanidin terminal subunits, the mean percentages of each compound were very similar to those for the parents, although more pronounced differences were found for the extension subunits, where the mean (−)-epicatechin value was lower than that of the parents; one hybrid contained only 40% of (−)-epicatechin and 56% of (−)-epigallocatechin. The mean percentage of (−)-epicatechin-3-*O*-gallate was lower than for Syrah grapes. For the white grapes, the average mDP was similar to that of red grapes but the terminal (+)-catechin proportion was higher, with three hybrids containing 100% of (+)-catechin. On the basis of discriminate analysis (data not shown), the percentage of terminal (+)-catechin allowed for the correct differentiation between red- and white-skinned grapes. However, the presence of 100% of terminal (+)-catechin is not related to white grapes alone because Pastor del Rio and Kennedy (*27*) have reported that (+)-catechin was the only terminal flavan-3-ol monomer observed in the skin of Pinot noir. Again, the presence of (−)-epigallocatechin extension subunits was higher than in the parents.

For red seeds, the variability found among the hybrids was lower, the mDP falling within the range of values detected for the parents (**Table 4**). The terminal subunit compositions were very similar to those found in Monastrell, and almost no difference in extension subunits was observed. The same applied to the seed from white grapes. Chira et al. (*17*) reported that the influence of variety on seed proanthocyanidin extracts was less significant than the effect of the vintage year, whereas the opposite was observed in the case of skin proanthocyanidin extracts.

Although no great differences were found between them, some hybrids bore grapes with a proanthocyanidin composition that could have some impact on the different characteristics of wines. Experimental evidence has shown that the mDP and galloylation of wine proanthocyanidins are important structural variables that affect wine astringency perception (*9*). In the current study, we found a positive correlation ($r^2$ = 0.60) between mDP and galloylation percentage, suggesting that hybrids bearing grapes with a very high mDP could impart higher astringency. However, Chira et al. (*17*) found that the correlation between mDP and astringency could be modulated by the presence of (−)-epigallocatechin. Some of our hybrids showed relatively high percentage of (−)-epigallocatechin (>50% of extension subunits). Fernández et al. (*22*) also found that the presence of (−)-epigallocatechin units in the proanthocyanidins of Carmenere grapes reduced the "coarse" astringency perception. On the basis of these studies, it is apparent that candidate hybrids having structural and quantitative features consistent with improved red wine astringency have been developed. Understanding the relationship between grape-based proanthocyanidins and their corresponding red wines is the subject of future studies.

One issue that could have an impact on the results discussed above relates to the extractability of proanthocyanidins during wine production and physiological factors that might be related to this. Proanthocyanidin extractability appears to be mainly dependent on the molecular size, with the mDP being much higher in the nonextracted fraction (*20*). To a lesser extent, the percentage of galloylation has been found to be higher in nonextracted compounds (*20*), and other studies have shown that the extraction of skin and seed proanthocyanidins may vary so much that the measurement of total proanthocyanidin in fruit is of little use in predicting the level of proanthocyanidin that can be expected in the wine (*19*). Therefore, these new lines are being evaluated for astringency quality with the knowledge that red wine astringency is dependent not only on grape proanthocyanidin structure and amount but also on extraction (*17*), polysaccharides (*29*−*31*), and acidity (*32*, *33*), among other parameters.

### Note Added after ASAP Publication

There was an error in the title in version of this paper published ASAP October 26, 2009; the corrected version published ASAP November 2, 2009.

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