



Viticulture, enology and marketing for cold-hardy grapes



Frontenac and Marquette Berry Skin and Pulp Characterization During Ripening

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Background and Rationale: The cold climate cultivars Frontenac and Marquette are complex hybrids that combine characters of the European wine grape (*V. vinifera*) and North American *Vitis* species. The ripening process and chemical and molecular character of many of the European cultivars are well described; however, these characteristics are not well described for the emerging cold climate cultivars. The objective of this study was to characterize the berry ripening processes in Frontenac and Marquette at the gene and chemical level, providing baseline information that may be used in combination with traditional Brix, pH and TA measures for identifying biomarkers for signature varietal characters and optimal ripeness.

Treatments:

- Frontenac and Marquette berries sampled at veraison, 20, 22, 24 °Brix.
- Wine made from 22, 24 °Brix.
- Frontenac, Marquette, La Crescent and St. Croix berries sampled for volatiles from veraison to harvest at weekly intervals.

Methods: (11 point Calibri bold) Include info about data collection, study management, etc. (11 point Calibri)

- Frontenac and Marquette transcriptome, metabolome and sensory analysis. In 2012 and 2013 berries were collected from NE1020 replicated trial at 5 timepoints from veraison to harvest. Four replicates were collected at each timepoint and pH, Brix, TA and seed maturation observed. RNA was extracted from all samples.
 - Transcriptome analysis was conducted using RNA sequencing (RNASeq) and comparisons on skin and pulp and cultivars conducted to identify differentially expressed genes to provide skin and pulp characters in berries at harvest.
 - Wine was made using UMN standard protocol at Prairieberry Winery, Hill City, SD.
 - Skin and pulp samples were provided to UMN for metabolite analysis (Hegemann lab). Whole berries were provided for sensory analysis (Vickers lab).
- Four replicates of single berry volatiles were collected at 5 timepoints for Frontenac, Marquette, La Crescent and St. Croix in SD. Volatiles were transferred to Somchai Rice at Iowa State University for analysis.
 - Four time points of Marquette and Frontenac berry skin and pulp RNA were sequenced (RNASeq) for transcriptome analysis of berry ripening profile.

Results:

- Berry development was monitored from veraison to harvest for pH, Brix and TA as well as seed maturity. Frontenac seed maturity was about 7 days slower than Marquette in both 2012 and 2013 (Fig 1) and harvest.

- Wines were made at one harvest point in 2012 and two timepoints 22 and 24 Brix in 2013 and were transferred to UMN for berry and wine sensory analysis.

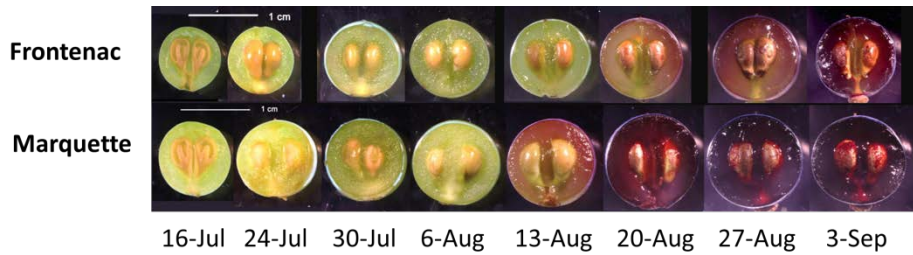


Fig 1. Frontenac and Marquette berry development monitored weekly from 30 days post bloom to 20°Brix.

- Gene expression was analyzed in pulp and berry at harvest. Three other time points (veraison, 20 and 24 Brix) are currently being analyzed. A comparison of pulp, skin and cultivars indicates gene expression in common and unique to tissue or cultivar (Fig 2). Skin samples show a greater number of genes expressed over pulp between the two cultivars. There are relatively equal numbers of uniquely expressed genes between cultivars.

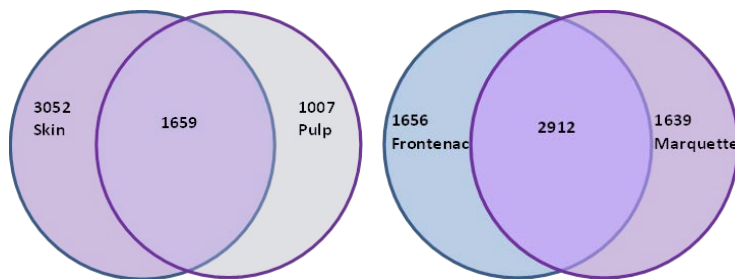


Fig 2. Numbers of differentially expressed (up or down regulated) genes between skin and pulp or between cultivars. Intersection of circles indicates number of genes that are modulated in common within a tissue or cultivar comparison.

- Stilbene synthase the enzyme involved in synthesis of the antioxidant resveratrol was expressed only in the skin and more stilbene synthase genes were expressed in Frontenac than Marquette.
- Although there were common patterns of flesh coloration during ripening in Frontenac and Marquette, there was greater expression of anthocyanin biosynthesis genes in Frontenac than in Marquette (Fig 3). This example verifies the gene expression results against the existing knowledge of the enhanced coloration of Frontenac juice and wines.

	Front Skin	Marq Skin
VIT_05s0062g00720	36	
VIT_12s0034g00080	47	
VIT_02s0033g00450	159	42
VIT_02s0033g00390	165	84
VIT_05s0049g01020	429	120
VIT_02s0033g00380	531	219

Fig 3. Significant expression of anthocyanin biosynthesis genes in Frontenac and Marquette berry skin. Front = Frontenac, Marq = Marquette and dark red indicates highest level of expression.

- Terpenoids contribute to the aroma and flavor of the berries and wine. Terpenoid biosynthesis genes were differentially regulated between skin and pulp and between cultivars with the greatest differences occurring in the berry skins. A comparison of pulp and skin indicated that genes upregulated in monoterpenoid biosynthesis predominated in the berry skin of both Frontenac and Marquette. There were also distinct differences in expression patterns between Terpenoid biosynthesis genes expressed in Frontenac and Marquette (Fig 4). Expression of genes in the taxol and menthol biosynthesis pathways were characteristic

of Marquette. Frontenac showed and upregulation of genes in the geranyl diphosphate biosynthesis pathway which is known to produce many volatiles such as those found pine resin.

- The ripening profile time course of gene expression in berry skins and pulp is ongoing and genes that contribute to the development of flavonoids, phenolics and other terpenoids are noted to be differentially expressed between Frontenac and Marquette.

	Front Skin	Marq Skin
VIT_12s0134g00030	0.0	8.5
VIT_06s0004g06480	0.1	2.0
VIT_17s0000g05580	0.2	1.4
VIT_01s0010g02320	0.5	2.5
VIT_13s0067g00380	0.6	0.1
VIT_15s0046g03600	0.8	1.1
VIT_13s0067g00370	0.8	0.2
VIT_19s0135g00200	1.9	4.9
VIT_00s0253g00140	1.9	0.4
VIT_19s0135g00190	2.4	5.2
VIT_05s0049g00400	3.1	1.4
VIT_15s0046g03570	3.1	1.2
VIT_08s0032g00240	5.0	2.4
VIT_15s0021g01060	6.7	3.2
VIT_11s0016g01290	7.4	1.2
VIT_19s0015g02500	9.1	1.9
VIT_17s0000g09610	13.3	1.7
VIT_15s0048g01490	22.3	4.1
VIT_02s0025g04880	119.5	35.3

Fig 4. Differences in monoterpenoid biosynthesis genes in Frontenac and Marquette berry skins. Front = Frontenac, Marq = Marquette and dark red indicates highest level of expression.