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An Exploratory Analysis of Yield, Fruit Composition, and Single Vine Wines of Interspecific Cold-hardy White Grapevine Breeding Lines

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Abstract

Grapevine production in North Dakota is limited by extreme weather events such as freeze events in late spring, early fall, and winter. The North Dakota State University Grape Germplasm Enhancement Project was established to screen, identify, and develop grapevines with potential for long-term success within North Dakota's environmental constraints. To increase understanding of production and enological trait relationships, single vine fermentations were conducted in the fall of 2018. Grapevine harvest metrics, fruit composition, and wines were analyzed using principal components analysis to reduce dimensionality. Multiple relationships identified in principal component analysis were explored for trends within breeding material; among them, negative linear relationships were observed between berry mass and total soluble solids. A similar negative linear relationship utilized in various crosses and warrant further consideration as part of breeding goals and selection criteria for North Dakota. This work captures a snapshot of hybrid grapevine breeding populations undergoing selection in the 2018 season and provides an exploratory overview of seedling trait variation through descriptive statistics and factor analytics. Continued selection and intercrossing among promising lines are anticipated to give rise to more consistent grapevine growing options for North Dakota farmers; extended and improved phenotyping and screening methods will enhance the effectiveness of selection.

Introduction

There is a growing interest from cool climate vineyards and wineries in the cultivation of grapevine (Vitis spp.) varieties that are adapted to North Dakota, and other cooler growing regions [1-3]. The University of Minnesota grapevine breeding program developed multiple wine grapes such as 'Frontenac', 'Marquette', 'La Crescent', and, most recently, 'Itasca' that have led to expansion of viticulture and enology in northern climates [1,3-9]. Growers in previously unexplored parts of the northern U.S. and Canada now produce commercial wines from cold-hardy wine grapes developed by the University of Minnesota, South Dakota State University ('Valiant'), Elmer Swenson ('Prairie Star', 'Brianna', 'Sabrevois', 'St. Croix', and more), and other private breeders [1,2,10,11]. These new cultivars have driven an increase in the number of cool climate plantings. However, North Dakota vineyards have struggled to consistently produce yields due to frequent, substantial winter dieback that has been observed to injure even typically cold-hardy grapevines such as 'Bluebell', 'Frontenac', 'Frontenac gris', 'Hasansky Sladky', 'John Viola', and 'King of the North' [3,12].

In 2010, the North Dakota State University Grape Germplasm Enhancement Project (NDSU GGEP) was established [3]. The goal of the NDSU GGEP is to assess available wine grape cultivars and generate new breeding lines with regional adaptation for North Dakota. The project has evaluated varieties and seedlings in the field and within growth chamber conditions to improve selection of parents [12-16]. As part of these efforts, seedlings from directed crosses are ultimately evaluated for their capacity to produce a crop with early fruit ripening parameters for North Dakota's short growing season [3]. Breeding targets include consistent yield (goal: >4.94 ton/ ha), relatively lower titratable acidity (TA) (goal: <12.0 g/L tartaric acid equivalents), and a reduction in herbaceous aromas often present in V. riparia derived hybrids. Acidity in wines is dependent on the region of production and the genetics of the grapevine cultivar. In North Dakota, most cultivars are harvested with a high TA level (frequently above 14.0 g/L) [17-19].

Canopy and yield management practices fail to substantially reduce the TA for many interspecific hybrid wine grapes, with the results often varying by year, cultivar, and location [17,19-22]. Producing a ripe crop in cold climates is an obstacle addressed extensively in the literature with various vineyard management approaches assessed including fruit zone leaf removal, crop thinning, shoot thinning, and trellis selection [17,19,23-29]. Yet, rather than struggling to manage vines with high acid, a more direct method to alter fruit composition of regionally grown grapevines may be to select breeding material that ripens early with a low TA and acceptable pH and total soluble solids (TSS) level [3]. Northern U.S. grown hybrids with V. riparia background frequently attain high levels of TSS [3,17,19,21,22]. Thus, one of the greatest challenges to regionally consistent wine production is identifying improved grapevine germplasm material that is high yielding with low acid content [18].

The aim of this study was to improve our understanding of variation and interrelationships between fruit composition, wine characteristics, and yield in the context of grapevine breeding populations for the purpose of cold-hardy, short-season, white wine grapevine selection.



Materials and Methods

Growing location

This experiment took place as part of active selection within the NDSU GGEP; as a result, vine age and parentage varied among evaluated grapevine seedlings. Grapevine seedlings generated as part of the NDSU GGEP were planted along with rooted vines of regionally important varieties at the North Dakota State University Agriculture Experiment Station located in Fargo, ND (46° 89' North, 96°81' West) between 2010 and 2016. Grapevines were planted in rows-oriented North to South as part of a seedling screening trial with 0.91 m between vines and 1.83 m between rows for seedlings planted in 2015 and 2016. For seedlings planted prior to 2015, the spacing was 0.31 m between vines and 1.83 m between rows. As part of the breeding process, grapevines were actively culled (cut to the ground level and subsequently excavated with shovels) throughout each year based on disease prevalence and relative cold hardiness. Supplemental water via dripline irrigation was only provided during the first two years of trunk establishment.

Individual grapevines were trained to one-to-two trunks using a bamboo pole fixed to a trellis wire 1.7m above the soil forming a high wire cordon training system. For initial screening and selection in years prior to this experiment, grapevines were trained to a unidirectional cordon. Following culling of vines previously described, retained vines were trained for an addition cordon; thus, they were modified to a bilateral training system.

Spring pruning was conducted to retain a maximum of 30 buds per vine, with vine cordons pruned to a maximum cumulative length of 2m. Due to vines' youth and variable freeze injury, bud number was frequently less than the maximum prescribed bud number. Minimizing vine spacing was critical in years 2013-to-2017 to maximize the number of individuals planted within research plots; however, by the time of this evaluation, vine spacing within row was increased to at least 2m by vine culling in prior years. No crop load management was applied beyond spring pruning. Sucker removal occurred late May prior to flowering and before netting the vines. Canopy management was conducted in mid-July as shoot combing immediately prior to manual application of bird nets.

The soil at the site is a poorly drained Fargo silty clay (fine, smecticic, firgid typic Epiaquerts) with a slope of 0-1% [30]. The climate is considered a humid continental climate (Köppen Dfa/Dfb) described as USDA plant hardiness zone 4a. Weather data were obtained from a North Dakota Agricultural Weather Network data collection site located approximately 500 m north of the trial [31]. Growing degree day (GDD) accumulation was calculated starting May 01 using 10 $^{\circ}\mathrm{C}$ as the base temperature of subtracted from the daily average temperature with minimum temperature limit set at 10°C and maximum temperature limit set at 30°C. The 30-year average for temperature, precipitation, and GDD accumulation was based on the period of 1991-2020.

Grapevines evaluated included internally developed seedling vines and check lines that were used as standards for comparison of relative hardiness, yield, and fruit composition when making selections (Table 1). Check lines were individual vines planted as rooted cuttings from regionally important breeding lines (ES 10-18-14, ES 3-20-33) or wine grapes ('St. Pepin', 'Adalmiina', 'Prairie Star', 'Frontenac gris').

	Cr	oss		Vines	Cross
Туре	Seed parent	Pollen parent	Name/ pseudonym	within cross/ individual (n)	exhibits V. <i>labrusca</i> traits
Breeding	ES 5-6-64	ES 3-16-21	ES 10-18-14	2	Yes
line	MN 78	Canadice	ES 3-20-33	1	Yes
	ES 114	5 114 Seyval blanc St. Pepin		1	Yes
Cultivar	ES 2-3-17	ES 35	Adalmiina	2	Yes
	ES 2-7-13	Alpenglow	Prairie Star	2	Yes
	V. riparia 89	Landot noir	Frontenac gris	2	No
	Beta	Somerset Seedless	-	1	Yes
	C-14	Alpenglow	-	4	Yes
	ES 4-23-60	MN 1131	-	1	Yes
Germ	Frontenac gris	Adalmiina	-	17	Yes
plasmz	MH.ND. 004.17	MH.ND. 004.17	-	2	No
	MN 1095	MN 1131	-	1	No
	MN 1131	Solaris	-	3	No
I Innomod	MN 1095	Espirit	-	1	Yes

Table 1: Type of vine (check line [breeding line or cultivar] or NDSU-GGEP germplasm), cross parentage, number of vines evaluated within cross, and whether vines from a cross exhibit V. labrusca traits.

Harvest metrics

The time of harvest was determined through weekly pre-harvest sampling of 10 berries per vine. Two berries were randomly selected per cluster from five clusters per vine to create pre-harvest samples. Pre-harvest samples were sealed in a labeled plastic bag and transported from the field to laboratory where they were analyzed on the day of collection. Pre-harvest samples were crushed and evaluated for TSS using a digital refractometer [Pal-1, Atago Co., Tokyo, Japan] and pH [Orion Star A111, ThermoFisher Scientific, Waltham, MA, USA]. The refractometer was calibrated with deionized water daily before use. The pH meter was calibrated daily before use via a three-point calibration with buffers of pH 4.01, 7.00, and 10.01.



Considering the nature of the breeding effort, with no preexisting knowledge of seedling grapevine harvest chemistry, harvest date was dictated by a target pH close to an optimal white wine pH. Harvest was conducted when pre-harvest sampling indicated a grapevine seedling's pH was in the range of 3.1 to 3.3; harvest was conducted one to five days after pre-harvest sampling.

At harvest, total cluster number and yield were recorded for each vine. Single cluster masses were calculated based on a representative three-cluster sample. Berry mass and diameter were monitored based on a random 15-berry sample derived from the three-cluster sample (5 berries per cluster: one from the top, three from the middle, and one from the bottom of each cluster). Harvest TSS and pH were determined as described for the pre-harvest samples, via a Pal-1 refractometer and Orion Star A1111 pH meter, respectively. The TA value were measured via manual titration of a 3 mL sample of juice with a 0.1N sodium hydroxide solution to an endpoint pH of 8.2 following dilution in deionized water using the same Orion Star A111 pH meter [32].

Wine Making

Following the collection of harvest metrics, grape berries from individual vines were manually removed from rachises by researchers wearing sanitary, low-odor, foodgrade nitrile gloves. Berries were placed into food-grade plastic bags and immediately stored in a walk-in cooler at 4°C for between one and four hours depending on the number of samples to be processed for each harvest date. Following completion of manual destemming for all genotypes, berries were manually crushed within sealed plastic bags starting with the first genotypes destemmed and finishing with the last, thus minimizing variation in time between processing procedures. Once berries were crushed, plastic bags were manually squeezed, extracting juice which was captured into argon purged clear glass bottles used for primary fermentation ranging in size from 375 to 3785 ml. Musts were immediately treated with 50 ppm of sulfur using a potassium metabisulfite stock solution, capped with a bung fitted with a three-piece fermentation airlock, and stored overnight (12hr) in a growth chamber set to 15°C.

One day after harvest and potassium metabisulfite addition, individual musts were inoculated with Saccharomyces cerevisiae yeast (EC-1118, Lallemand Inc., Montreal, Québec, CAN) at a rate of 0.264g/L following rehydration. Rehydration of yeast was conducted at 37 °C for 20 min in a solution containing a 1:1.25 ratio of yeast to-yeast rehydration nutrients (Go-Ferm^{*}, Lallemand Inc., Montreal, Québec, CAN).

One day after inoculation, fermentation was visually confirmed based on activity of yeast and release of carbon dioxide. After confirmation of successful inoculation, 0.264g/L of a yeast nutrient supplement were added (Fermaid * K, Lallemand Inc., Montreal, Québec, CAN). Individual musts were allowed to complete primary alcoholic fermentation at 15° C (for most musts this occurred 14-21 days after inoculation). Completion of primary fermentation was assessed visually by slowing of yeast activity and precipitation of solids from wines. After primary fermentation completion, wines were manually transferred from their gross lees via pouring through a layer of sanitized cheesecloth in a stainless-steel funnel into argon purged, sanitized, clear glass containers fitted with bungs and airlocks in a volume smaller than the vessel utilized previously in primary alcoholic fermentation. Wines were allowed an additional 28 days to complete any final fermentation in the smaller vessel with reduced head space. Finally, wines were sealed with plastic tasting corks and cold stabilized at 4°C for a minimum of 2 weeks. Wines were then stored in their containers until spectral evaluation.

Wine evaluation

Wine pH and wine TA were measured as described for harvest; however, a 5 mL sample of wine was utilized for titrations. Multiple spectral properties of wines were measured to obtain an understanding of wine composition. Assessment of color intensity (A420 nm + A520 nm), color hue (A420 nm/A520 nm), and estimates of total phenols (A280 nm - 4), total hydroxycinnamates (A320 nm - 4), and total flavonoids ([A280 nm - 4] – [{0.66} × {A320 nm - 1.4}), were conducted using a 1 mm

path length quartz cell measured in a UV-Vis spectrophotometer (GenesysTm 10S UV-Vis Spectrophotometer, ThermoFisher Scientific, Waltham, MA, USA); values were adjusted to a path length of 10 mm as described by Iland et al. [33]. CIELab color coordinates were calculated with MSCV* software [34], obtaining values for lightness (L*), chroma (L*), hue (h), red-green (a*), and yellow-blue (b*) based on measurements collected from undiluted samples in 10 mm pathlength polymethyl methacrylate UV-cuvette cells (UV-Cuvette semi-micro, BrandTech* Scientific, Inc., Essex, CT, USA).

Statistical analysis

Descriptive summary statistics were calculated using R software version 3.6.1 [35]. Descriptive statistics were calculated separately for checks and seedlings to allow anecdotal preliminary assessment of germplasm performance prior to replication of vines. Further exploratory data analysis such as principal component analysis and regression screening only focused on grapevine seedlings for germplasm under selection. Principle component analysis was conducted using the factoextra v1.07 and prcomp function of the stats v3.6.2 package [36,37]. Color data for samples was processed with the colorspace package [38]. Where CIELAB color coordinates are used as an aesthetic to color within geom_point, the depicted color is based on the L*, a*, and b* values for each wine sample. Figures were created using the ggplot2 package [39].

Results

Growing conditions

The preceding dormant season (assessed for Oct. 2017 to May 2018) had an absolute minimum temperature of -31.4°C, with 18 days below -25°C, and only three days with minimum temperatures below -30°C (Table 2). A total of 180 days at the site had temperatures below 0°C during the period.

Table 2: Accumulated GDD and precipitation during the growing season for research plots at the North Dakota State University Agriculture Experiment Station, located in Fargo, ND, 2018.

Mandh	Number of Days Minimum Temperature Below							
Month	0°C	-15°C	-25°C	-30°C	Min temp (°C)			
Oct. 2017	9	0	0	0	-9.8			
Nov. 2017	29	2	0	0	-16.5			
Dec. 2017	31	14	7	2	-31.4			
Jan. 2018	31	19	7	1	-30.2			
Feb. 2018	28	21	4	0	-26.5			
Mar. 2018	29	3	0	0	-18.9			
Apr. 2018	21	2	0	0	-16.1			
May-18	2	0	0	0	-1.6			
Total	180	61	18	3				

The final spring frost event of 2018 occurred on May 11 (-1.6°C) and did no observable damage to buds in the plots. The 2018 growing season had 140 frost free days before the first fall frost occurred on Sept. 28 (-0.5°C); this came 22 days after the final white wine grape genotype was harvested for fermentation screening as part of this experiment. By the first harvest date, Aug. 16, a total of 1161 GDD were accumulated (Figure 1). The following two harvest dates on Aug. 24 and 31 had greater GDD accumulation of 1242 and 1305 GDD. The final harvest date on Sept. 06 had a total of 1353 accumulated GDD. There was a 46% decrease in rainfall in May compared to the 30 yr average (Figure 2).





Figure 1: Accumulated growing degree days (AGDD [calculated with a base of 10 °C]) during the growing season for research plots at the North Dakota State University Agriculture Experiment Station, located in Fargo, ND in 2018 compared to the 30 yr average.



Figure 2: Accumulated precipitation as rainfall during the growing season for research plots at the North Dakota State University Agriculture Experiment Station, located in Fargo, ND in 2018 compared to the 30 yr average.

Summary statistics

Berry diameter ranged from 1.10 to 1.67 cm among check lines and 1.14 to 1.63 among seedling germplasm evaluated (Table 3). Berry mass had a minimum value of 0.62 g and a maximum value of 2.85 g for check lines and ranged from 0.62 to 2.65 g for germplasm. The mean berry mass was 1.63 g for the white grape germplasm. Cluster mass, a function of berry number and berry mass, had a mean of 97.8 g for check lines and a mean of 97.7 g for germplasm (Table 4). The minimum seedling cluster mass of 10.70 g was considerably smaller than the maximum value of 192.00 g. Yield for germplasm followed in a wide range, between 0.11 kg to 4.92 kg per vine with a mean of 1.90 kg and a median of 1.39 kg.

 Table 3: Summary statistics for berry characteristics of white wine grapevine germplasm (seedlings) and checks grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018.

	meter (cm)	Berry I	Mass (g)	
Value	Checks	Seedlings	Checks	Seedlings
Min Value	1.1	1.14	0.83	0.62
Max Value	1.67	1.63	2.85	2.65
CV (%)	16.14%	13.53%	44.95%	32.76%
Variance 0.05		0.04	0.55	0.25
Standard Deviation	0.21	0.19	0.74	0.53
Mean 1.33		1.39	1.65	1.63
Median	1.27	1.33	1.22	1.57

 Table 4: Summary statistics for cluster and yield characteristics of white wine grapevine germplasm (seedlings) and check lines grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018.

	Cluster	mass (g)	Yield (kg)	
Value	Checks Seedlings		Checks	Seedlings
Min Value	65.6	10.67	0.58	0.11
Max Value	171	192	3.37	4.92
CV (%)	30.98%	52.07%	43.49%	71.05%
Variance	919.07	2174.62	0.63	1.84
Standard Deviation	30.32	49.84	0.79	1.35
Mean	97.85	95.71	1.83	1.9
Median	86.67	89.33	1.87	1.39

Fruit accumulation of TSS for seedlings had a mean of 20.7°Brix for the germplasm, and 19.8 for the check lines (Table 5). The highest observed TSS was 25.8 and the lowest was 14.9, both for the germplasm. Must pH values at harvest were between 3.19 and 3.61 for check lines and 3.15 and 3.66 for germplasm. The TA at harvest for germplasm had a mean of 10.19 g/L tartaric acid equivalents while check lines averaged 10.93 g/L. Must TA values were between 16.00 and 5.50 g/L across both groups. Wine TA values were between 5.50 and 11.70 g/L (Table 6). The mean pH of wines was 3.14 for check lines and 3.16 for germplasm.

Table 5: Summary statistics for must total soluble solids, pH, and titratable acidity (TA) for white wine grapevine germplasm (seedlings) and check lines grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018.

	Must TSS (Brix)		Must pH		Must tarta equiv	TA (g/L ric acid valents)
Value	Checks	Seedlings	Checks	Seedlings	Checks	Seedlings
Min Value	16.7	14.9	3.19	3.15	5.5	6.75
Max Value	23.4	25.8	3.61	3.66	15.1	16
CV (%)	11.97%	12.64%	3.48%	4.69%	22.89%	22.01%
Variance	5.62	6.48	0.01	0.03	6.26	5.8
Standard Deviation	2.37	2.63	0.12	0.16	2.5	2.24
Mean	19.81	20.77	3.33	3.38	10.93	10.19
Median	18.5	20.65	3.3	3.39	11.5	10.25

Table 6: Summary statistics for titratable acidity (TA) and pH of wine for white wine grapevine germplasm (seedlings) and check lines grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018.

	Wine pH		Wine TA (§ equi	g/L tartaric acid ivalents)
Value	Checks	Seedlings	Checks	Seedlings
Min Value	2.98	2.81	7	5.5
Max Value	3.41	3.76	11	11.7
CV (%)	3.67%	7.56%	14.57%	15.13%
Variance	0.01	0.06	1.58	1.92
Standard Deviation	0.11	0.24	1.26	1.21
Mean	3.14	3.16	8.63	8.01
Median	3.12	3.13	8.2	8

Citation: Svyantek A, Wang Z, Stenger J, Theisen N, Brooke M, Auwarter C and Valenti HH (2023) An Exploratory Analysis of Yield, Fruit Composition, and Single Vine Wines of Interspecific Cold-hardy White Grapevine Breeding Lines. Arch Agri Res Technol 4: 1050

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Total phenolics for germplasm wine samples averaged 2.91 absorbance units (AU) (Table 7). This was higher than either the hydroxycinnamates (1.58 AU) or the flavonoids (1.87 AU) mean values for seedlings, and higher than the mean values observed for check lines. The highest color density observed for seedlings was 0.96 AU while the mean color density was 0.32 AU (Table 8). Color hue values were between 1.29 and 11.00 for germplasm wines and 2.13 to 8.10 for check lines. Wines varied in the color intensity, with a germplasm mean of 0.21 AU, minimum of 0.02 AU, and maximum of 0.54 AU.

Table 7: Summary statistics for total phenolics, hydroxycinnamates, and flavonoids of
white wines from grapevine germplasm (seedlings) and check lines grown at the North
Dakota State University Agricultural Experiment Station, Fargo, ND in 2018.

	Total j	phenolics AU)	Hydroxy (/	oxycinnamates (AU) Flavonoid		oids (AU)
Value	Checks	Seedlings	Checks	Seedlings	Checks	Seedlings
Min Value	0.73	0.91	0.10	0.20	0.01	0.04
Max Value	4.23	7.37	2.58	4.48	2.82	4.69
CV	58.39%	50.81%	73.22%	69.31%	75.28%	53.86%
Variance	1.51	1.62	0.54	0.95	1.12	0.91
Standard Deviation	1.23	1.48	0.74	1.09	1.06	1.01
Mean	2.10	2.91	1.00	1.58	1.40	1.87
Median	1.67	2.53	0.88	1.29	1.32	1.73

 Table 8: Summary statistics for color attributes of white wines from grapevine germplasm (seedlings) and check lines grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018.

	Color de	Color density (AU) Color hue (AU) Color hue (AU)		Color hue (AU)		Color intensity (AU)	
Value	Checks	Seedlings	Checks	Seedlings	Checks	Seedlings	
Min Value	0.08	0.03	2.13	1.29	0.08	0.02	
Max Value	0.25	0.96	8.1	11.00	0.18	0.54	
CV	34.96%	73.53%	55.25%	80.83%	23.90%	65.43%	
Variance	0.00	0.04	5.75	7.08	0.00	0.01	
Standard Deviation	0.06	0.23	2.4	2.65	0.03	0.14	
Mean	0.18	0.32	4.34	3.28	0.14	0.21	
Median	0.22	0.28	2.67	2.19	0.15	0.19	

Wines were relatively light in color based on their L* value, with a mean of 97.9 for check lines and 95.87 for germplasm (Table 9). Wines were generally neutral regarding their a* value, with a seedling range of -2.70 to 2.45 The samples also tended towards yellow, based on the b* values, with a germplasm mean of 9.07. The L* and h* angle showed high variance and standard deviations (Table 10).

Table 9: Summary statistics for CIELAB color space traits (L*, a*,b*) of white wines from grapevine germplasm (seedlings) and check lines grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018.

	Light	Lightness (L*) Red-green (a*) Yellow-blue (b		Red-green (a*)		-blue (b*)
Value	Checks	Seedlings	Checks	Seedlings	Checks	Seedlings
Min Value	97.3	87.9	-0.73	-2.7	-1.58	2.37
Max Value	98.3	99.3	3.74	2.45	8.5	19.3
CV	0.39%	3.44%	4120.69 %	1612.63 %	46.86%	46.25%
Variance	0.14	11.32	1.76	1.14	8.04	16.84
Standard Deviation	0.38	3.3	1.33	1.06	2.84	4.19
Mean	97.9	95.87	0.03	0.07	6.05	9.07
Median	98	97.25	-0.45	-0.25	6.35	7.8

Table 10: Summary statistics for CIELAB color space traits (C* and H*) of white wines from grapevine germplasm (seedlings) and check lines grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018.

	Chro	ma (C*)	Perceived hue (H*)		
Value	Checks	Seedlings	Checks	Seedlings	
Min Value	4.06	2.37	90.59	81.75	
Max Value	8.32	19.45	337.1	106.9	
CV	17.25%	46.34%	63.37%	6.05%	
Variance	1.31	17.19	5854.09	30.57	
Standard Deviation	1.14	4.23	76.51	5.52	
Mean	6.63	9.12	120.75	91.27	
Median	6.6	7.83	94.91	91.92	

Principal Component Analysis

The first two principal components combine to account for approximately 51 percent of the variation among samples (Figure 3). The L* and a* values lay negatively opposed, with h* and color hue closely aligned with L*. Yield was coupled with cluster number, single cluster mass, and vine age. Harvest TSS was negatively associated with berry size traits (mass and diameter).







Figure 3: Loading plot of principal components 1 and 2 for harvest characteristics and subsequent white wines of grapevine germplasm grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018. Abbreviations: a= a* (green-red), b= b* (blue-yellow), CI= color intensity (AU420), C= L* (chroma), CD= color density, CH= color hue, cl.n= cluster number, Flav= flavonoid content, fv.ml= fermenter volume, h= hue, H.GDD= harvest growing degree days, h.pH= must pH, h.SSC= must TSS, h.TA= must TA, Hydroxy= hydroxycinnamate content, L= L* (lightness), Phenolics= total phenolics, sbd.cm= single berry diameter, sbm.g= single berry mass, scm.g= single cluster mass, vine. age= age of vines, w.pH= wine pH, w.TA= wine TA, yld.kg= single vine yield.

PC3 only accounted for 13 percent of variation and all remaining PC accounted for less than 7 percent individually (Figure 4). PC1 was driven by yield, fermentation volume, berry diameter, berry mass, cluster number, cluster mass, and L*, a*, and h* traits. PC2 was driven by a combination of hydroxycinnamate and phenolic content, as well as wine color intensity, color density, b*, L*, and wine pH. PC3 was shaped almost entirely by acid parameters of the must and wine along with phenolics, flavonoids, cluster number, single cluster mass, and vine age.



Figure 4: Scree plot (A) and trait contributions to PC1 (B), PC2 (C), and PC3 (D) for harvest characteristics and subsequent white wines of grapevine germplasm grown at the North Dakota State University Agricultural Experiment Station, Fargo, ND in 2018 (red, dashed line indicates significant contributions). Abbreviations: a= a* (green-red), b= b* (blue-yellow), CI= color intensity (AU420), C= L* (chroma), CD= color density, CH= color hue, cl.n= cluster number, Flav= flavonoid content, fv.ml= fermenter volume, h= hue, H.GDD= harvest growing degree days, h.pH= must pH, h.SSC= must TSS, h.TA= must TA, Hydroxy= hydroxycinnamate content, L= L* (lightness), Phenolics= total phenolics, sbd.cm= single berry diameter, sbm.g= single berry mass, scm.g= single cluster mass, vine.age= age of vines, w.pH= wine pH, w.TA= wine TA, yld.kg= single vine yield.

Linear relationships

Multiple linear relationships were examined in greater detail to inform breeding decisions (Figure 5). Phenolics and flavonoids had a strong linear relationship (R= 0.90; p <0.001). Single cluster mass was closely related to yield per vine (R= 0.76; p <0.001). Conversely, single cluster mass was negatively related to TSS (R= -0.42; p = 0.021). Single berry mass was also negatively related to TSS (R= -0.54, p = 0.021). Phenolics and color intensity were positively related (R= 0.44; p= 0.016). Similarly, hydroxycinnamates were related to color intensity in samples (R= 0.63, p <0.001).



Figure 5: Examination of linear relationships within white wine grapevine seedling germplasm among (A) phenolics and flavonoids in wine, (B) single cluster mass and yield, (C) single cluster mass and total soluble solids, (D) phenolics and color intensity, ℓ hydroxycinnamates and color intensity, and (F) Single berry mass and total soluble solids. Individual sample points are colored based on the specific CIELAB color coordinates of wines.

Discussion

Breeding lines influence observations

This study assessed variation within the NDSU GGEP, but due to small and unequal population sizes was only able to examine linear relationships regarding phenotypic correlations. While phenotypic correlations are not equal to genotypic correlations, genotype contributes substantially to the phenotypic correlations observed among traits [40-43]. This data set was representative of NDSU GGEP breeding lines and was conducted as part of an active breeding program during selection. As a result, vine fruit that was fermented represented promising breeding lines that captured the variation of vines which have survived North Dakota conditions and were not previously culled due to unacceptable traits. Many of the evaluated relationships were confounded with factors such as vine age, harvest date, survival, selection, and genetic backgrounds of grapevines, thus there is a clear need for refinement of methodology if any comparisons are to be made directly across these confounding factors or for the purpose to identify genetic variance within the program. Within larger vine and wine breeding data sets with reduced confounding factors generated from future work, phenotypic correlations may approach genetic correlations [44-46]. As the number of vines evaluated increases, more informed selection can be conducted based on the local understanding of these correlations in the context of plant development, physiology, and breeding program goals with economic weighting of traits for selection [46-51].

Many high-yielding white genotypes in the NDSU GGEP have substantial amounts of *V. labrusca* in their parentage background. For the evaluated breeding lines approximately 63% of the crosses exhibit *V. labrusca* traits. This number increases to 80% of the total evaluated seedlings, with over half of the evaluated material coming from a single cross. This distribution is akin to the check lines, with five out of six unique check genotypes exhibiting *V. labrusca* traits (all but 'Frontenac gris'). From our internal observations, *V. labrusca* parentage frequently contributes to relative

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increases in berry size (mass and diameter), decreases in TSS, increases in pH, and decreases in TA; this is similar to overall observations in cultivar evaluation [3]. Some of these anecdotal observations were supported by this work, such as the strong linear relationship between single berry mass and TSS in *V. labrusca* derived material (Figure 6). These characteristics (berry size, low acid) derived from *V. labrusca* backgrounds are desirable traits for phenotypic selection. However, selection of *V. labrusca* characteristics in yield components and fruit composition may also increase *V. labrusca*-based fruit aromatic compounds that vary in consumer acceptance [52-55].



seedling germplasm between single berry mass and total soluble solids (left= germplasm without *V. labrusca* traits; right= germplasm exhibiting some level of *V. labrusca* traits). Individual sample points are colored based on the specific CIELAB color coordinates of wines.

Foxy aromas are often attributed to V. labrusca backgrounds and are frequently given a negative connotation [56-58]. Yet, in North Dakota, with limitations of growing season length and pressure from severe winter events, it may be logical to favor greater yield components and lower TA over fruit aromatic compounds when conducting selection, especially in early generations of breeding efforts.

Vine age and environmental influence

Environmental influence on fruit and wine composition are critical factors requiring further assessment. The age of vines was closely correlated with yield (R= 0.72; p<0.001) as would be expected in a breeding plot of variably planted vines maturing amid selection (Figure 7). This confounding factor supports the need to evaluate distinct plantings separately for selection and culling purposes as vine age influences many characteristics of vine performance [59-61]. Furthermore, in a population of vines, such as the seedlings examined here, there is a negative relationship between GDD accumulation and some fruit composition traits, such as TSS (Figure 8). This is contrary to what would be expected under normal viticultural conditions and in climatological models in which a longer or hotter growing season, and thus greater GDD accumulation, is typically associated with greater values for ripeness parameters [62-64]. This counter-intuitive relationship observed likely stems from compounded effects of vine age, crop load, and history of cold damage; thus, GDD accumulation may only be useful for screening and selection purposes within specific planting years. Growing season length as monitored by GDD and frost-free days frequently are driving factors in fruit quality, especially in North Dakota [18]. However, GDD relation to ripening time early in a vine's life may not be an adequate predictor of later GDD requirements for seedlings. Future work tracking yield components and fruit composition in a diverse population of grapevines across multiple years, from seedling to mature yielding vines in the absence of selection would provide useful data to inform breeder decision making related to the effect of vine age on final attributes.







Figure 5: Linear relationship within white wine grapevine seeding germplasm between harvest growing degree days (GDD) and total soluble solids. Individual sample points are colored based on the specific CIELAB color coordinates of wines.

For grapevine breeding programs considering combining data across vine age, years, populations, plots, harvest dates, or planting locations, analytical techniques that utilize consistent check lines planted across years and throughout plots are important. Examination of selection methodologies must consider techniques from other perennial fruit crops as well as tree breeding [65-68]. The most efficient number of check lines should be explored to ensure the best use of breeder resources. A greater number of check lines for hardiness (some checks cold susceptible others extremely cold hardy) and ripening times (early, medium, and late ripening) planted at regular intervals within all seedling plots may be necessary to help breeders deduce cold tolerance and potential fruit quality of seedlings across ages of vines. Yet, for the purposes of resource optimization and applied germplasm selection in an environment such as North Dakota, with frequent cold damage events, the amount of selection weight placed on grapevine survival compared to fruit composition traits should be carefully evaluated [12,69].



Improving small scale fermentation methods

Due to the scale and nature of these single vine fermentations, multiple factors that remain important to wine composition were incompletely addressed. These factors include temperature of fermentation, pressing conditions, enzymatic extraction, and yeast strain. Color, examined within this study, is one of the most important sensory qualities for wine, and numerous factors can influence wine color, such as grape composition, fermentation method, and storage conditions [70]. Macroclimate, mesoclimate, and fruit microclimate all have major impacts on wine quality, such as color and aromas [71].

Genetic variability for berry coloration within the breeding program may have contributed to variation observed in L*, a*, CD, and CI values, amongst other traits monitored spectrophotometrically. Grape berries may be classified into blanc, gris, red, and noir color categories. However, at the early stages of breeding an individual seedling grapevines' color variability is not known, much like their optimal pH, TSS, and TA values. For the purposes of NDSU GGEP wine production, grapevines were designated into two primary categories at harvest, white wine, or red wine. From that point on, their fermentation procedures were set. Blanc, gris, and some red grapes fall into the white wine category. While under normal production circumstances it is possible to produce white wines from even noir grapes, under the small-scale fermentation and processing conditions described here it was possible that excess color may have bled into the must. This may happen either during the temporary cold soak period between berry removal from rachises and pressing or during the actual berry pressing procedure itself. Refinement and streamlining of processing methods will improve insight into genetic variability for wine color in white wine germplasm. Means to standardize or expedite must extraction may include uniform freezing of berries, which has been examined for red musts [72,73]. Alternatively, small-scale presses or enzymatic extraction methods may be used to standardize juice processing and remove the human element. Further methods to increase consistency are discussed in the following section on fermentation scale [74-76].

Grape, must, and wine temperature affect color throughout fermentation and through cold stabilization, with temperature altering the precipitation of color pigments [70]. White wines are normally fermented at lower temperatures than red wines to preserve aromatic compounds. The fermentation temperature (15°C) utilized in this study was considered relatively low and may have influenced fermentation rate and final wine characteristics. High concentrations of esters have been demonstrated at low temperatures [77]. Both glycerol and ethanol were differentially correlated to fermentation temperatures as well [78].

Beyond temperature, pressing conditions greatly influence the initial must characteristics. High quality pressing of wines allows for white wine musts with minimum polyphenols content and with low oxidation levels. Vertical presses exert pressure up and down, and horizontal membrane presses exert pressure from filling a membrane with compressed air or water [79]. The pressing procedures normally include progressive extraction with a slowly increased level of pressure that avoids crushing the solid parts of the clusters while limiting enzymatic activity that might contribute to oxidation. Semi-open membrane and closed membrane presses were tested for Listán blanco wine [79]. Total acidity of the wine was decreased with increased pressure, but sugar content of the must was not different in the two systems. Thus, pressing systems can influence must composition based on what components are pressed from grape berries. The amounts of distinct aromatic substances in the pressing fraction increased with increasing pressure in three systems examined; meanwhile, the formation of ethyl esters and acetates in the corresponding wines were also increased with pressure [80].

Pressing conditions have also been shown to affect the phenolic composition of the musts and wines in V. vinifera white wines, with increased pressure resulting in higher polyphenol content and radical scavenging power in a comparison among free-run must, light pressed must, and heavy pressed must [81]. Heavy pressed musts presented a higher browning index. Compounds such as the polyphenols, glutathione, and antioxidant properties were affected by the amount of pressure applied during grape pressing in Sauvignon Blanc must [82]. Plus, pressing fractioning could cause polysaccharide and oligosaccharide wine composition changes [83]. Thus, numerous compounds may be influenced by pressure of pressing, Furthermore, there is a high potential for cultivar-pressing condition interactions to be influenced by maturity, berry mass, seed size, and the characteristics of the mesocarp, exocarp, and endocarp of berries. In our examination, a standardized, manual pressing method was utilized; these techniques may be altered to improve aroma and reduce phenolic extractions in future breeding material assessment as higher yields become available for fermentative evaluation. The natural pectolytic enzymes (already in grapes) and the commercial enzymes (normally from Aspergillus niger) occur in three general types: pectin esterase, pectin lyases, and polygalacturonase. These three types of enzymes degrade pectin from the must through hydrolysis, further affecting the flavor of wine [84]. They are added at maceration, or for clarification of the must or wine [85]. Commercial maceration enzymes composed primarily of pectinases, cellulases, and hemicellulases enhance the maceration stage, increasing red wine color, tannin, and proanthocyanin extraction [86]. Likewise, glycosidase activity also takes place in improving the varietal aromas of the wines [87]. These products also behave differently under various temperature, pH, and ethanol regimes [88]. Neither native enzymes nor added enzymes were examined in this study, but they may be used beneficially in targeting aromatic compounds or color profiles in subsequent evaluations.

Clarity is critical for the quality of wine, but white wine can develop undesirable color during storage and aging. Among the color deterioration types, browning is a frequent phenomenon [89]. Browning gives rise to increased color intensity, decreased brightness, and heightened browning index [90]. Total phenolic and flavanol concentration are linked with wine antioxidant capacity, but the type of phenolics and flavanols also vary with different stages of storage period; the higher the concentrations of monomeric hydroxycinnamic and gallic acid, the larger the trend for wine to become brown [91].

Almost all phenolic compounds decrease due to oxygen addition, especially the hydroxycinnamic acid derivatives in white wines. Oxygenation could cause white wine browning, and hydroxycinnamic acid and sulfur compounds could also increase browning [92]. Pre-fermentative skin maceration might improve wine quality due to the extraction of phenolic and volatile compounds. Skin maceration and hyperoxygenation combined techniques might also be used for their different effects on color and aromas, depending on fermentation process and grapes [93-95]. Salacha's results indicated that the total phenolic and flavanol concentration were significantly correlated with the browning rate constants, specifically, the browning development was predominately associated with flavanols [96]. Enzymatic browning may occur frequently at the early stages of winemaking, where hydroxycinnamic esters were playing the main role, and non-enzymatic oxidation was more related to flavanol content [94]. Both hydroxycinnamates (non-flavonoids) and flavan-3-ol (flavonoid) contribute to white winemaking. Flavonoids mainly exists in the skin, seeds, and stems, instead of berry pulps, therefore, the flavonoids in our research wines may be influenced by harvesting method, press cycles, and pressures. A plausible contributor, crushing temperature, has been shown to influenced the rate of polyphenol extraction during alcoholic fermentation [97].

Numerous challenges in small-scale fermentations lead to minimal product for evaluation, minimal taster input, and limited shelf-life of wines. For these reasons, scale of fermenter (375 mL to 3875 mL) may influence the final wines. Future experiments of this kind should standardize fermentation methods to the minimum volume available, ideally setting a yield threshold prior to harvest. Likewise, to account for potential berry characteristic influence on pressing, additional samples should be taken at harvest of berries and must for examination of phenolic compounds. Furthermore, the influence of harvest date should be examined in breeding lines by utilizing a standardized minimum fermentation scale. Numerous factors are influencing white wine fermentation properties, to account for all of them at such a small-scale is not possible; however, future fermentations may improve and streamline the methodology using pre-fermentative maceration with enzymes to increase juice yield, identification of ideal fermentation conditions for other cold-hardy wine grapes, or development of a microscale pressing procedures that removes human error.

Conclusion

This work examined vines and wines from breeding material within the NDSU GGEP that were evaluated as part of selection in the 2018 season. Significant linear relations were identified that may benefit breeding decision making, but due to the confounding effects of vine age and population, these relationships require further evaluation before application in selection. Grapevine genetic backgrounds are important to consider when making selection, and further work must examine how to most appropriately weight traits for proficient and accurate selection of superior plant material. It may be advisable to conduct future selection separately for sub-classes of germplasm, such as those vines with or without V. labrusca traits. For future single vine wine fermentations conducted as part of breeding and selection, there are clear needs for refinement of fermentation methods to reduce experimental error that may be influenced by vine yield and fermentation volume.



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