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Sensory Analysis to Inform Breeding Decisions in a Segregating Grapevine Population

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Abstract. **In plant breeding settings, the flavor and aroma of new cultivars are traditionally evaluated based on the judgements of breeders or small technical groups without using data from sensory panels. A sensory descriptive analysis is one of the primary sensory evaluation techniques used to discriminate products. Because of time and monetary costs, descriptive analysis methodology has not been explored in grape breeding to advance the selection of target traits associated with the eating experience. We explored sensory evaluation methodology for screening grape (*Vitis* spp.) seedlings of a mapping family and generating quality sensory data for further genetic studies. In 2018, we developed a lexicon with 29 sensory attributes, including five aroma attributes, three basic taste attributes, and 19 flavor attributes. Participants were able to characterize differences in 26 of the attributes among the genotypes tested. Malic acid (MA) and titratable acidity (TA) were positively correlated with sourness, citronella, lime, lemon, green apple, and kiwi flavors. Total soluble solids (°Brix) were positively correlated with sweetness, aroma intensity, flavor intensity, and floral flavor. Sweetness was positively correlated with overall aroma, taste, flavor intensities, floral and Concord aromas as well as floral, fruity, and Concord flavors. A hierarchical cluster analysis separated the genotypes into seven distinct groups, including cluster 1, which included most genotypes with low flavor and aroma intensities for all attributes, and cluster 7 with individuals with the herbaceous/green flavor and aroma. The most promising cluster genotypes for table grape breeding were included in cluster 6. They were characterized by fruity, floral, and labrusca ('Concord') attributes. The methodology developed for this study can be exploited in plant breeding research to characterize the variation of flavor and aroma traits in mapping families.**

Grape flavor is determined by a combination of taste, aroma, and mouthfeel attributes. The balance of sugars and acids determines taste, while aroma is driven by a diverse array of volatile compounds that interact with sugars and acids to shape the overall flavor perception. Flavor and aroma are important commercial traits of table and wine grapes, but they have been neglected in many breeding programs because of the complexity of improving these traits. Instead, grape breeding programs focus on production traits such as disease resistance, berry size, shape, postharvest, and other agronomic traits not associated with the consumer eating experience (Klee 2010). The advancement of these traits happens for the following reasons: a few genes control these traits; genetic gain can be achieved faster; and screening methods are more standardized and established.

Traditionally, grape flavor evaluations rely heavily on analytical metrics, including total soluble solids (TSS; in °Brix), titratable acidity (TA), and maturity index (MI; TSS/TA). These metrics provide essential information about sweetness and sourness, but they fail to capture the full complexity of flavor and aroma as perceived by humans. As a result, most grape cultivars currently available on the market now have a combination of high sugar with reduced acid content and lower flavor and aroma profiles (Ubeda et al. 2020).

The challenges associated with flavor improvement in breeding programs are not unique to grapes. Similar issues have been observed in other crops such as tomato (*Solanum lycopersicum*) and strawberry (*Fragaria* ×*ananassa*), for which the emphasis on production traits had led to the decline of consumer-relevant qualities (Colantonio et al. 2022; Fan et al. 2021, 2022; Gilbert et al. 2015; Klee and Tieman 2013; Porter et al. 2023; Tieman et al. 2012).

Unfortunately, there is no standardized methodology for identifying, quantifying, and improving the flavor for cultivar development; consequently, breeders mainly assess flavor based on TSS and TA content, which are indicators of maturity that focus solely on compounds related to sweet and sour tastes. Instrumental methods cannot completely mimic human sensory responses or perceptions of food (Lawless and Heymann 2010).

A sensory descriptive analysis offers a solution to these challenges by providing a comprehensive and objective description of sensory perception in qualitative and quantitative terms (Murray et al. 2001). It can be performed with a relatively small group of highly trained participants using a standardized list of descriptors known as a lexicon. While a descriptive analysis is widely used in food science, its application in fresh fruit breeding programs, including table grapes, has been minimal and largely limited to wine grapes (Douglas et al. 2001; Findlay et al. 2007; Mansfield and Vickers 2009; Parish-Virtue et al. 2021). Moreover, the few studies that applied sensory methodology have primarily included tomatoes, strawberries, and blueberries (Bai and Lindhout, 2007; Casals et al. 2018; Colantonio et al. 2022; Fan et al. 2021, 2022; Gilbert et al. 2015; Klee and Tieman 2013; Oliver et al. 2018; Tieman et al. 2006).

Unfortunately, the descriptive analysis has not been well-explored to guide grape breeding programs because of the number of genotypes that require testing, the high cost per sample, and the amount of fruit available to conduct this study (Luby 1991). The descriptive analysis can be a valuable tool for selecting and improving or even maintaining flavor based on consumer-driven flavor profiles (Klee 2010).

In general, flavor and aroma phenotypic evaluations are conducted by the breeder or a small group of people who make selections through informal tastings that can be influenced by personal preferences rather than standardized sensory evaluation methods (Bowen and Grygorczyk 2021; Colantonio et al. 2022). This is because of sample limitations with one plant per unique genotype, the costs of conducting sensory evaluations, and the complexity of traditional sensory methodology (Bowen and Grygorczyk 2021). Sensory evaluation in the field may be limited by evaluator fatigue because the breeding

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Table 1. Sensory lexicon that defines the aroma and flavor attributes of the GE1337 grape mapping population. A working definition and reference samples were provided to panelists.

Attribute	Definition	Reference sample
Overall intensity		
Aroma	Overall intensity of the aroma	1–12 butanol scale
Taste	Overall intensity of taste	1–20 citric acid scale
Flavor	Overall intensity of flavor	1–20 citric acid scale
Aroma and flavor attributes		
Pineapple	Fruit aroma and flavor associated with fresh pineapple	3-cm piece of fresh pineapple (skin off)
Apricot	Fruit aroma and flavor associated with fresh apricots	A piece of fresh apricot with skin
Peach	Fruit aroma and flavor associated with fresh white peaches	A piece of fresh white peach with skin
Green plants	Green aroma and flavor typical of fresh grass	*Riparia* grape berries
Floral	Sweet fragrant aromatic associated with flowers and perfume	Muscat grape berries
Grape jam	Welch's grape jelly	Sample of Welch's grape jelly
Fruity	Sweet, intense flavor associated with a combination of mixed fruit, pineapple, melon, apple, grape	Combination of mixed, cut fresh fruit
Concord	Traditional grape flavor	Concord grape berries
Strawberry candy	Aroma associated with artificial grape flavor or artificial strawberry	LaetaFood Arcor strawberry candy
Green apple	Fruit aroma/flavor associated of green apples	Fresh green apple pieces
Grass	Unripe aroma characterized by cut grass	*Riparia* grape berries
Green pepper	Aroma/flavor associated with fresh green peppers	A piece of fresh green bell pepper
Basic taste descriptors		
Sweetness	Taste stimulated by sucrose and other sugars	5.0% sucrose in distilled water (25 g/500 mL)
Sourness	Taste stimulated by acids, such as citric, malic, phosphoric, etc.	0.075% citric acid in distilled water (0.375 g/500 mL)
Bitterness	Taste stimulated by tonic water	Market Pantry™ tonic water

team may taste hundreds of genotypes each day, including those that may be under-ripe, highly acidic, astringent, or bitter. Furthermore, thorough phenotyping of flavor and aroma attributes may only happen during the late stages of the breeding pipeline when the promising selections are evaluated for cultivar release and commercialization in replicated plantings. While a few breeding programs have begun to focus on improving grape flavor through advanced selections, the absence of early sensory screening and robust methodology for evaluating flavor and aroma limits the potential for genetic improvement.

The use of lexicons reduces the noise in sensory data by providing a product-specific list of attributes with definitions and references for panelists who may have different understandings and interpretations of the same descriptor based on their prior experiences. A useful lexicon supports clear and effective communication among panelists to characterize a wide array of sensory attributes in each sample. The number of descriptors in a lexicon depends on the product being described (Belisle et al. 2017; Bowen et al. 2019; Corollaro et al. 2013; Du et al. 2010; Suwonsichon 2019). However, the time and expense required to train and monitor the performance of individual panelists over extended periods (days or even weeks) have limited the use of a descriptive analysis for plant breeding (O'Sullivan et al. 2011).

Researchers who use a sensory descriptive analysis must focus attention on the presentation and ordering of samples for panelists. In a straightforward descriptive analysis experiment, panelists typically assess all samples and treatments. Tasting many samples during a single session may lead to adaptation and/or carryover effects that can alter panelist perceptions. Because of the many samples that are typically studied in a mapping population, it is almost impossible for all panelists to test them during a single tasting session. Similarly, in many fruit crops like apples, peaches, citrus, and grapes, fruit within a family may ripen across a long harvest season of several months.

Experimental designs such as the balanced incomplete block design and Latin square design offer a practical solution for managing large sample sizes ($n > 50$) and minimizing order and carryover effects without losing any statistical power (Ball 1997; Osafo 2020; Toutenburg and Shalabh 2009), thus making descriptive analyses more feasible for breeding programs.

Integrating the sensory analysis into the breeding pipeline has the potential to transform the development of new cultivars by predicting consumer preference and aligning with breeding targets. Hampson et al. (2000) emphasized that releasing a new cultivar without knowing consumer preferences for a given fruit increases the risk of market failure. To succeed, plant breeders must consider both growers' and consumers' current and emerging needs and desires to determine priority traits.

In recent years, the fruit industry, especially the table grape industry, has shifted from a commodity market to more value-added and branded options (Bowen and Grygorczyk 2021). Many grape breeding programs have studied, improved, and introduced new aromatic cultivars in the last decade. Likewise, the few reviews published have focused exclusively on basic chemistry metrics (TSS, TA, and MI) or berry metabolite characteristics despite the importance of understanding consumer perceptions of these metrics to guide breeding targets. Therefore, a systematic analysis of sensory perception associated with basic chemistry metrics and metabolite composition of the grape berries can facilitate the development of new cultivars that will fulfill the gap in the current breeding pipeline that does not have robust methodology to screen for flavor in grape populations.

Having a sensory evaluation tool in the breeding toolbox provides valuable information to support fruit breeders when selecting novel cultivars to achieve marketplace success. Several researchers have reported using sensory tools to assess fruit sensory attributes to support breeding decisions (Bowen and Grygorczyk 2021; Carneiro et al. 2020; Colantonio et al. 2022; Hampson et al. 2000; Kyriacou and Rouphael 2018; O'Sullivan et al. 2011; Suwonsichon 2019). However, most of these works have not demonstrated a tool that empowers a plant breeding program to develop an in-house sensory protocol. Therefore, the current work aimed to test a detailed and complete protocol for a grape sensory analysis performed by a trained panel for a large number of sample genotypes, examine the relationship between instrumental chemical measurement and sensory intensity measurements of grape berries in a segregating family of cold-hardy grapes. We used this method to investigate the sensory attributes found in grape berries from a cold-hardy mapping family to cluster samples with similar phenotypes to better understand the genetics and later inform the marker-trait genetic analysis.

Materials and Methods

Lexicon development

In 2018, six panelists who worked for the University of Minnesota grape breeding program met at the Horticultural Research Center and evaluated 29 genotypes from the GE1337 population to develop the lexicon,

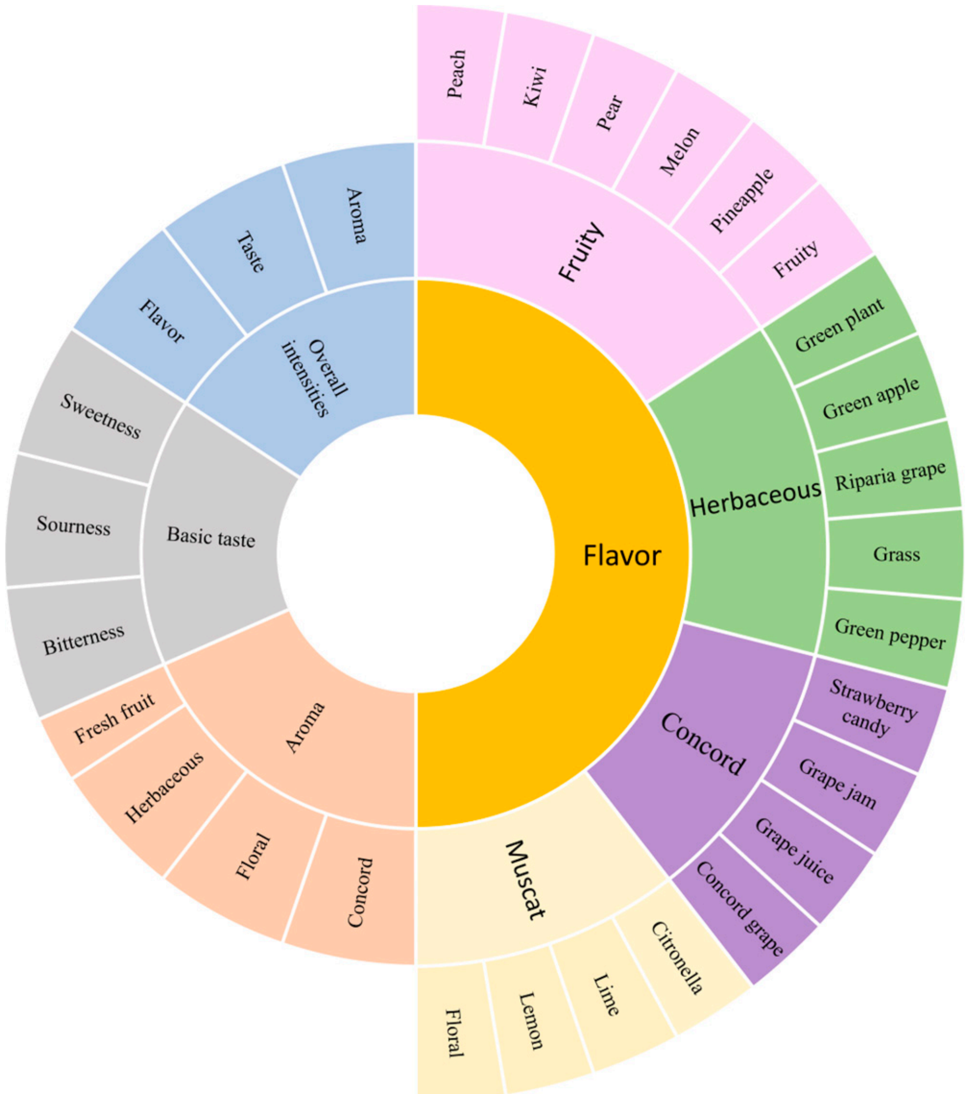

Fig. 1. Flavor and basic aroma wheel based on the lexicon developed in 2018 for population GE1337.

which is a list of terms, to describe the sensory properties typical of grape berries. Five of the six panelists had previous experience with the sensory evaluation of grapes and wines. All participants received samples containing five berries (from the 29 genotypes) in a small clear zip-top bag, which they first opened and smelled; then, they tasted the grape berries to describe the aroma and flavor. Participants drank water between samples to wash their mouths to eliminate residue from the previous sample. Then, panelists discussed the best word to describe each flavor and aroma attribute detected. A list of 29 sensory attributes was determined (Table 1), including overall flavor and aroma (orthonasal perception), basic taste, and specific flavor (retronasal perception). The aroma of samples referred to the scent or fragrance produced by grape berries. Taste referred to the sensory experience when panelists ate the grape sample with a nose plug (plastic foam nose clips; Frienda, Wuhan, China). Flavor referred to the sensory experience when panelists ate the grape sample without a nose plug.

A wheel with all attributes was created using the information collected in 2018 (Fig. 1). The selected attributes included in the lexicon aligned with the list of the attributes used to describe flavors of fresh berries and wine of 'La Crescent' and 'Frontenac' (Brady 2017; Del Bel 2014; Mansfield and Vickers 2009).

Panelist training

The number of panelists varied across the study (25 participants in 2019; 30 participants in 2020). All participants volunteered to attend 1 d of training and 4 d of tasting sessions. Panelists did not receive any compensation for participating in this study. Institutional Review Board approval for using human subjects was granted for all sensory panels. Panelists were assigned to one of the five (2019) or six (2020) groups, where every group was assigned to a Latin square design with its own set of sample genotypes and a common control to all groups.

A training session was performed to reduce variations among panelists in the sensory analysis. The training was offered during two sessions and locations (Horticultural Research Center or Saint Paul, MN, USA). Panelists were trained to evaluate grapes using a modified version of a sensory panel procedure developed by Del Bel (2014). Panelists also received a set of 20 citric acid standardized calibration scales (https://sensorycenter.cfans.umn.edu/calibrated-scales-used-umn-sensory-center) representing intensity levels from 0 to 20 and 12 butanol samples representing 0 to 12 intensity levels (ASTM International 2018) in 30-mL cups with lids. Panelists were asked to sample all citric acid and butanol standards to gain familiarity with the intensity scales. They were directed to use the citric acid scale as a reference for ratings for taste and flavor evaluations and the butanol scale to rate aroma intensities. Using these calibrated scales allowed us to compare intensity ratings across sessions and years.

Once familiarized, panelists received a set of five randomized citric acid samples labeled with three-digit codes as a calibration exercise (intensity levels of 0, 3, 7, 8, and 10) and were asked to assign an intensity score of 0 to 20 to each sample. The citric acid standards were available, and panelists were encouraged

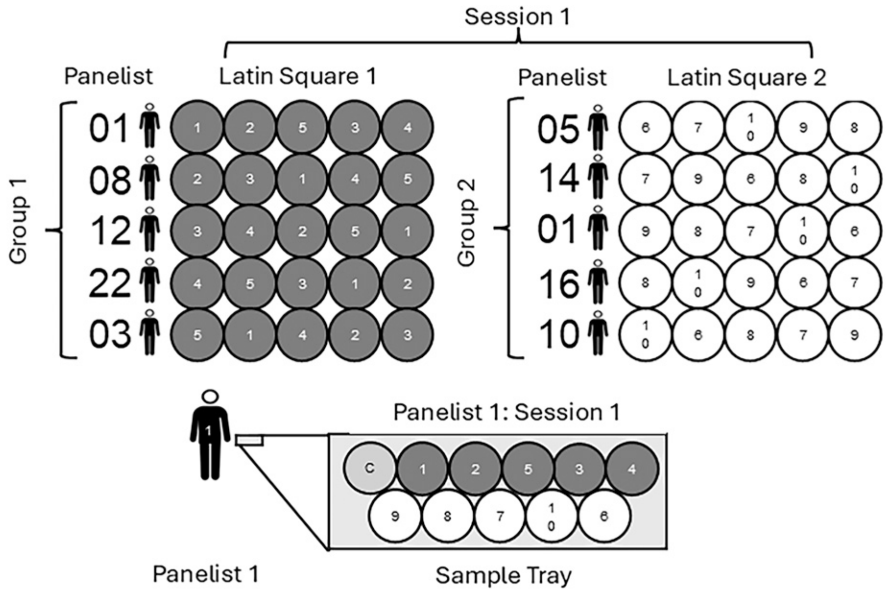

Fig. 2. Sensory experimental design scheme for assigning approximately 110 samples to 30 panelists using Latin square designs over two replicated sessions (1 and 3; 2 and 4). Each panelist was randomly assigned to two groups. Each group had its own Latin square design with a unique set of five samples for sessions 1 and 3 and sessions 2 and 4. Therefore, each panelist evaluated 10 samples during a session plus a control (C). Panelists evaluated the replicated samples during sessions 3 and 4, but samples were presented in a different order by reshuffling within each Latin square. An example sensory evaluation tray for panelist 1 during session 1 is shown.

to use the reference scale to help with their evaluations. Next, panelists were asked to rank a set of four samples of sweet and salty tastes from 1 to 4 (low to high) for perceived sweetness. Then, panelists received two samples of Mott's apple juice (100% and 15% dilute apple juice) and were asked to rank the samples based on the sweetness of each sample.

The salty samples were made with table salt with different concentrations. Participants received a tray with flavor references based on the lexicon (Table 1). They smelled and tried flavor identifiers to familiarize themselves with attributes that might be present in grape berry samples from hybrid grapes. Grape samples that typified wild species and hybrids included muscat (high terpene content), labrusca ('Concord' and artificial grape flavors typical of *Vitis labrusca*), herbaceous (common in *V. riparia*), and neutral (low aromatic compounds).

Finally, panelists practiced techniques for smelling and tasting the grape berries. Using a 1-oz cup with five berries, panelists evaluated the berries in the following manner: opened the lid; pressed one berry; closed the lid; waited for 1 min; opened half the lid and smelled it to evaluate the aroma (orthonasal odor); plugged the nose and placed one grape berry in the mouth, chewed it, and rated the sample for basic tastes (sweetness, sourness, bitterness); unplugged the nose, placed one or two grape berries in the mouth, chewed, and rated the sample for flavor attributes. Panelists used the citric acid scale as a reference to rate the basic taste and flavor attributes and the butanol scale for aromas.

Fruit production, harvest, and sample preparation

Fruits from self-rooted grapevines with approximately 5 years of growth were assessed to evaluate their sensory and chemistry attributes. A grape mapping population (GE1337 based on sample availability: n = 92 in 2019 and n = 103 in 2020) derived from 'Itasca' ('Frontenac gris' × MN 1234) × MN 1250 ('La Crescent' × MN 1198) was used for the study. A total of 103 unique genotypes were tested over the 2 years. Of these, 19 samples were tested only during one year (4 in 2019 and 19 in 2020). The data analysis included all samples from both years, ensuring a comprehensive assessment of the sensory attributes. This family was selected because of its expected segregation for labrusca, riparia, and muscat flavor profiles based on the pedigree and ancestor performance. Vines were grown at the University of Minnesota Horticultural Research Center, Excelsior, MN, USA (lat. 44°52′08.1″N, long. 93°38′17.3″W), spaced 0.8 m within rows and 3.0 m between rows, and trained on a top-wire, high-cordon system (Domoto et al. 2016). The clusters (grape bunches) did not receive any gibberellic acid treatment, thinning, or nutrient treatment to increase berry size or improve fruit quality. Because the vineyard is part of a breeding program evaluation plot, minimal spray treatments were used for disease and insect control. Parents and grandparents were evaluated each year.

Every year at harvest, a sample of approximately 40 berries per genotype representing three to seven clusters was taken and pooled to assess the TSS content of the clusters from each genotype in the family. The TSS content in berries was tested using a 0 to 32 °Brix temperature-compensating refractometer (Atago Corporation, Tokyo, Japan). Clusters were harvested when the sample reached a minimum level of 22 °Brix. Grape clusters used for the sensory panel were harvested on 8 Sep 2018 (lexicon development only), from 6 to 17 Sep 2019, and from 27 Aug to 11 Sep 2020. After harvest, samples were stored in 1-gallon ziptop bags (Ziploc®; S.C. Johnson & Son, Inc., Racine, WI, USA) in a walk-in cooler at 0 °C.

Two days before sensory evaluation, grapes were removed from cold storage, and five berries from three to 10 clusters (the number of clusters depended on the yield of each vine) were randomly selected; a small piece of the pedicel was kept. Samples were visually inspected to assist in selecting berries with uniform ripeness based on color. Five berries of each genotype were placed into a 1-oz plastic sampling cup and topped with a lid (Comfy Package, Brooklyn, NY, USA). Cups were labeled with three-digit randomized codes. The cups were stored at 4 °C until the training or sensory tasting sessions. All cups for that day were removed from the cooler 1 hour before the training or testing to reach room temperature.

Eleven samples (10 sample genotypes from the population and one control sample 'Itasca') were presented in the labeled cups; they were arrayed in two rows with five samples in the top row and six samples in the bottom on Cup Tray-30 Well trays (Frontier Agricultural Sciences, Newark, DE, USA). The sample presentation per panelist was a layout based on four randomized 5 × 5 Latin square designs, allowing the sample to be presented to each panelist at a unique position (Fig. 2) (Williams 1949). Two unique Latin square designs were used for each session.

'Itasca' berries served as a control sample. The genotype 'Itasca' was pooled from the same berries sample, harvested, and stored on the same day. However, the parental sample genotype 'Itasca' cups were served to a specific group of panelists, while the control sample was served to all panelists in the study.

Sensory evaluation

Panelists would self-calibrate their aroma and flavor intensity palate by assessing one unknown butanol (aroma) and two unknown citric acid (flavor) samples before each tasting session started. Panelists assessed the calibration samples and recorded the answers on the survey instrument, and their responses were verified for accuracy. If a panelist's answers were correct, then that panelist could continue the sensory evaluation. If the panelist did not answer correctly, then that panelist was informed of the correct answer and asked to retest the calibration samples. This step was performed to help panelists familiarize themselves with the reference calibration scales and improve consistency with the intensity measurements across 4 d of tasting, 2 years of the study, and 25 to 30 different panelists.

Table 2. Analysis of variance (ANOVA) of sensory (flavor and aroma) attributes of the GE1337 population. Main fixed effects were genotype, year, group session. The results show significant differences among all attributes during the 2 years of studies. The mean ± standard error (*SE*) of each attribute is listed in the table. Sensory attributes were tested as dependent variables. Genotype, year, group, and session were modeled as fixed effects. Panelist and replicate were modeled as random effects. The group effect was not shown because it was not significant, except as noted for one attribute. The random effects are not shown in this table because panelist was significant for all attributes ($P < 0.001$) and replicate was nonsignificant for all attributes, unless noted.

Attribute	Mean ± *SE*	Genotype	Year	Session
Overall intensity				
Aroma	5.6 ± 2.7	***	***	*
Flavor	8.4 ± 3.3	***	***	NS
Taste	7.3 ± 3.2	***	***	NS
Aroma attributes				
Fresh fruit	3.0 ± 2.6	***	NS	NS
Floral	2.1 ± 2.2	***	***	**
Herbaceous[i,ii]	2.9 ± 2.5	***	NS	NS
Concord	1.1 ± 2.0	***	*	NS
Taste attributes				
Sweetness	4.9 ± 2.9	***	**	NS
Sourness	4.9 ± 3.5	***	NS	NS
Bitterness	1.1 ± 1.7	NS	***	NS
Flavor attributes				
Floral	3.2 ± 2.9	***	*	NS
Lemon	2.7 ± 3.0	***	***	NS
Lime	1.9 ± 2.6	***	***	NS
Citronella	1.1 ± 2.2	**	***	*
Green pepper	1.0 ± 1.8	***	*	NS
Grass	1.6 ± 2.1	***	*	NS
Riparia grape	1.7 ± 2.4	***	***	NS
Green apple	2.7 ± 2.9	***	**	NS
Green plants[iii]	1.8 ± 2.3	***	NS	***
Concord grape	2.0 ± 2.9	***	NS	NS
Grape juice	1.9 ± 2.8	***	***	NS
Grape jam	1.2 ± 2.2	***	**	NS
Artificial strawberry	1.1 ± 2.1	***	NS	NS
Fruity	3.2 ± 3.0	***	**	NS
Pineapple	1.6 ± 2.3	*	**	*
Melon	1.7 ± 2.4	NS	**	NS
Pear	1.4 ± 2.2	NS	NS	NS
Kiwi	1.8 ± 2.5	***	**	NS
Peach	1.1 ± 2.0	**	***	NS

[i] Main random effect of panelist was significant at $P < 0.01$.
[ii] Main fixed effect of group was significant at $P < 0.01$.
[iii] Main random effect of replicate was significant at $P < 0.05$.
NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

After calibration, panelists received a tray with 10 cups containing five berries of each genotype and one control cup ('Itasca'). They were instructed to evaluate the cups in the order served to conserve the Latin square design and follow the tasting protocol practiced during the training session. Water was served during the sessions, and panelists were encouraged to cleanse their palates between tasting. Each panelist participated in four testing sessions.

A digital data collection tool was developed on QualtricsXM (www.qualtrics.com) in 2019 to improve speed, accuracy, and data analyses where panelists used a scale with 0 to 20 markings to indicate their response. This methodology facilitated data collection in 2020 during COVID-19, when panelists evaluated the grapes from their homes and recorded their answers online. The survey used in this research can be accessed using the following link: https://umn.qualtrics.com/jfe/form/SV_a8CDIh4xacXCY8C?Q_CHL=qr.

Each panelist tasted 20 different sample genotypes and four control samples over four tasting sessions (4 d). Panelists were organized into groups of five and randomly assigned to subsets of genotypes using a Latin square design (Fig. 2). On days 1 and 3, panelists evaluated the same genotypes, and on days 2 and 4, they received a second set of genotypes. For the replicate days, the samples were reshuffled within each Latin square. The control was positioned differently on the tray according to the day of the tasting session (first, fourth, sixth, and last sample).

Fruit chemistry

To measure TSS and total titratable acidity (TA), 30 to 50 berries per individual (depending on the vine's yield) were pressed to produce a 50-mL aliquot sample. The TSS was assessed using a hand refractometer (MISCO Palm Abbe PA201 Portable Digital; MISCO, Solon, OH, USA). The TA was determined using a 1:4 solution of juice in deionized water, titrated with a 0.1 mol/L NaOH solution, and expressed as $g{\cdot}L^{-1}$ tartaric acid with the Metrohm 916 Ti Touch® auto-titrator (Metrohm, Herisau, Switzerland), and pH was measured with the same instrument. Malic acid (MA) was analyzed using a subset of juice with an L-Malic Acid Assay Kit (Megazyme Ltd., Wicklow, Ireland).

Experimental design and data analysis

The experimental design used a balanced incomplete block design and Latin square design to manage a large number of samples. Panelists were organized into groups and randomly assigned to subsets of samples, ensuring each genotype was assessed by multiple panelists while minimizing order and carryover effects. Using a 5 × 5 Latin square design, five panelists were randomly grouped to taste the same group of five genotypes. Each panelist participated in four tasting sessions, where samples were presented randomly within each session. The four-session structure was designed to balance panelist fatigue and optimize sensory evaluations, with each panelist evaluating a subset of genotypes and a common control to maintain consistency across sessions.

The variation in intensity of a given attribute in each genotype was assessed using a univariate mixed analysis of variance for each of the 29 sensory descriptors studied (Table 2). For the model, sensory attributes were tested as dependent variables. Fixed effects were year, genotype, session, and group. Random effects were panelist and replicate. The analyses were performed using RStudio (R version 3.6.1; R Core Team 2019) using lme4 version 1.1-26 and lmerTest version 3.1-3 (Bates et al. 2015; Kuznetsova et al. 2017). In addition, a hierarchical cluster analysis was conducted using Hmisc and ggdendro packages, followed by a dendrogram analysis to explore the relationship among genotypes based on the sensory attributes and chemistry data averaged for 2 years. The number of optimal clusters to conduct the hierarchical cluster analysis was determined using the R package factoextra (version 1.0.7). First, a hierarchical cluster and dendrogram analysis of the parents and grandparents was conducted as an initial step to characterize the potential diversity of flavor and aroma in this family. Pearson correlations using sensory and chemistry data were calculated using the Hmisc R package version 4.5-0.

By using a balanced incomplete block design and Latin square design, we aimed to accurately capture the variation in sensory traits while accounting for environmental and panelist effects. This approach provided a comprehensive framework to assess sensory attributes, thus ensuring the data could inform future genetic studies and support breeding decisions.

Results

This study aimed to test the grape sensory evaluation protocol by evaluating the main effects of the experimental design. Genotypes, the main effect expected to vary in the population, were perceived differently, with statistically significant variations in most attributes, with labrusca (fruity, Concord aroma and flavor, grape jam, and juice) and herbaceous (green pepper, riparia grape, grass, green plants) emerging as the predominant flavors

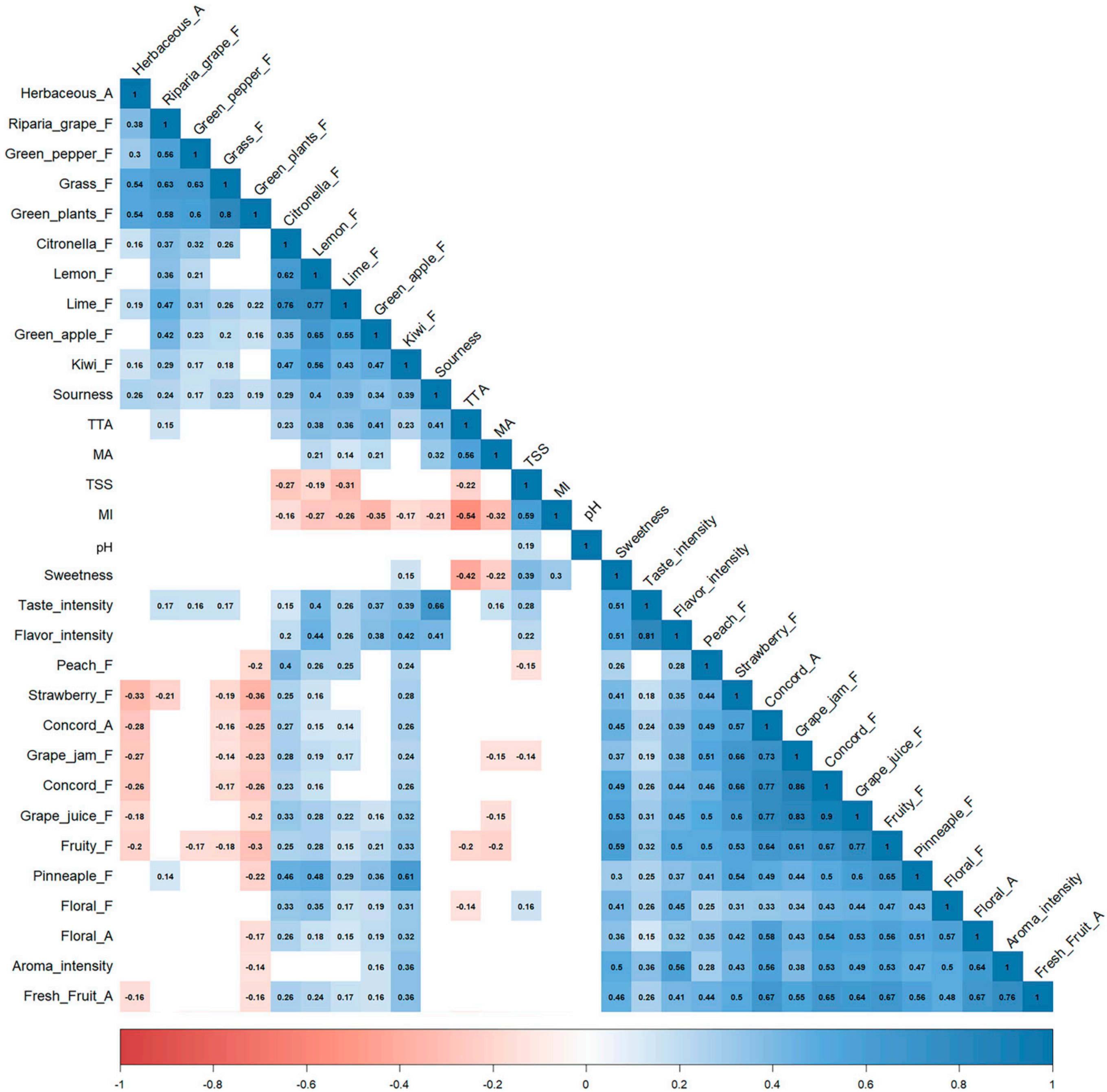

Fig. 3. Pearson correlation coefficients showing relations between basic chemistry metrics and sensory attributes from the flavor (trait_F) and aroma (trait_A) wheel. The analysis was performed using average data collected from berries of all genotypes collected in 2019–20. Only significant correlations are shown ($P < 0.05$).

and aromas. However, the overlapping of strong intensities of herbaceous and labrusca attributes was not perceived in any samples. The panelists identified fewer samples with overlapping floral and labrusca flavors and aromas. Using our experimental design resulted in a descriptive analysis of each sample, and the hierarchical cluster analysis grouped genotypes into seven distinct classes based on those profiles. The panelists identified three sensory attributes (bitterness, melon, and pear were not statistically significant among the samples). However, the year effect was significant for bitterness and melon (Table 2).

Year effect was statistically significant for the overall intensity of the aroma, overall intensity of flavor, overall intensity of taste, and multiple aroma and flavor attributes (Table 2). The panelists rated all sensory attributes higher in 2019 than in 2020, except for overall intensities of aroma and taste, floral herbaceous and Concord aromas, sweetness, sourness, and floral, green pepper, grass, green apple, green plants, and Concord grape (Supplemental Tables 2 and 3).

The main effect of the session was not significant for most of the descriptors used in this study, except for five attributes (overall intensity of aroma, floral aroma, citronella, green plants, and pineapple).

The panelist effect was significant for all attributes evaluated, but sample replicates did not influence any attributes tested. However, the repeatability of the panelists' performances within and among groups showed significant effects for herbaceous aroma within groups and for some attributes across sessions. A control sample ('Itasca') was used to evaluate group and panelist performance over four sessions, and the hierarchical cluster analysis showed the control sample clustered with the parent, 'Itasca', sample as expected (Fig. 4; Supplemental Table 1).

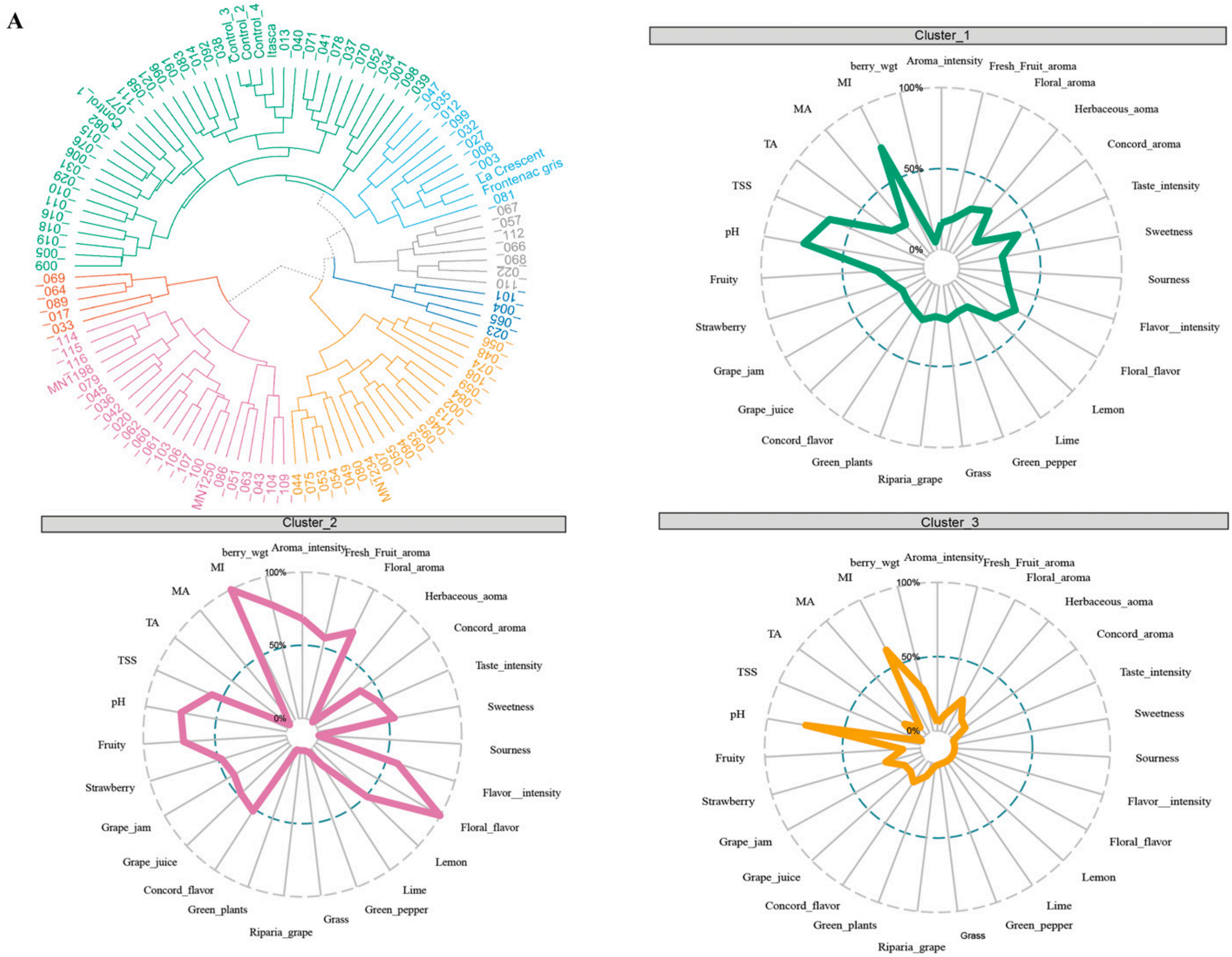

Fig. 4. (**A**) Dendrogram of the hierarchical cluster analysis (HCA) of descriptive analysis means from all genotypes tested in 2 years. The control sample is also the parent, 'Itasca'. The same color represents the cluster and spider plot. (**B**) Spider plots of the sensory descriptors for the samples from each HCA cluster across the 2 years of studies from all genotypes tested.

Correlation among basic chemistry properties and sensory perception

As an indicator of ripeness, the mean TSS and TA values across all samples were 23.45 ± 2.32 °Brix and 8.52 ± 1.71 g/L, respectively. The Pearson correlation analysis among the sensory attributes and the basic chemistry properties are shown in Fig. 3. The results revealed that taste and flavor intensities were positively correlated with sourness ($r = 0.66$ and 0.41), sweetness ($r = 0.51$ and 0.51), and TSS ($r = 0.28$ and 0.22).

Sweetness perception correlated positively with TSS ($r = 0.39$) and the maturity index (MI) ($r = 0.30$). In contrast, the sweetness correlated negatively with TA ($r = -0.42$) and MA ($r = -0.22$). Sourness perception correlated positively with TA ($r = 0.41$) and MA ($r = 0.32$). A negative correlation between sourness and MI ($r = -0.21$) was observed in this study (Fig. 3). Notably, sweetness showed positive correlations with several aroma attributes (floral, $r = 0.36$; fresh fruit, $r = 0.46$; and Concord, $r = 0.45$) and flavor attributes (floral, $r = 0.41$; peach, $r = 0.26$; fruity, $r = 0.59$; pineapple, $r = 0.3$; artificial strawberry, $r = 0.41$; grape jam, $r = 0.37$; Concord, $r = 0.49$; and grape juice, $r = 0.53$).

Several flavor attributes correlated positively with sourness, including herbaceous aroma ($r = 0.26$), riparia grapes ($r = 0.24$), green pepper ($r = 0.17$), grass ($r = 0.23$), green plants ($r = 0.19$), citronella ($r = 0.29$), lemon ($r = 0.40$), lime ($r = 0.39$), green apple ($r = 0.34$) and kiwi ($r = 0.23$). In contrast, herbaceous flavor attributes (green pepper, riparia grape, grass, green plants) showed negative correlations with the labrusca attributes (fruity, Concord aroma and flavor, grape jam, and juice) (Fig. 3).

Sensory intensity among descriptors that belong to similar categories were highly correlated ($r > 0.30$). For example, the components of the herbaceous category were positively correlated, including riparia grape, green pepper, grass, green plant flavor, and herbaceous aroma. The same trend was observed with citrus flavor, which showed a positive correlation between lime, lemon, and citronella attributes. Likewise, fruity, strawberry, Concord aroma, grape jam, grape juice, Concord flavor, and grape juice were positively correlated (Fig. 3).

Flavor and aroma characterization of the GE1337 population

The hierarchical cluster analysis revealed seven distinct clusters characterized by significant sensory attributes, and the dendrogram illustrates the genotypes within each cluster (Fig. 4). Cluster 1 ($n = 36$) exhibited no dominant flavor or aroma attributes, indicating that grape breeders might consider seedlings in this cluster as having a neutral profile (Fig. 4A). Cluster 2 ($n = 23$) featured a blend of moderate levels of fruity, strawberry, labrusca flavor (grape juice, Concord flavor, and grape jam), and floral aroma attributes coupled with a lower perception of sourness (Fig. 4A). Individuals in cluster 3 ($n = 21$) exhibited high pH and MI coupled with lower intensities of fruity, strawberry, and floral flavor attributes (Fig. 4A).

Cluster 4 ($n = 11$) included genotypes featuring the highest levels of TA and MA content, resulting in a flavor perceived as sour with predominant lemon and lime notes (Fig. 4B). Clusters 5 ($n = 7$) and 7 ($n = 4$) included seedlings exhibiting green plants, grass, riparia hybrid flavor, and herbaceous aroma

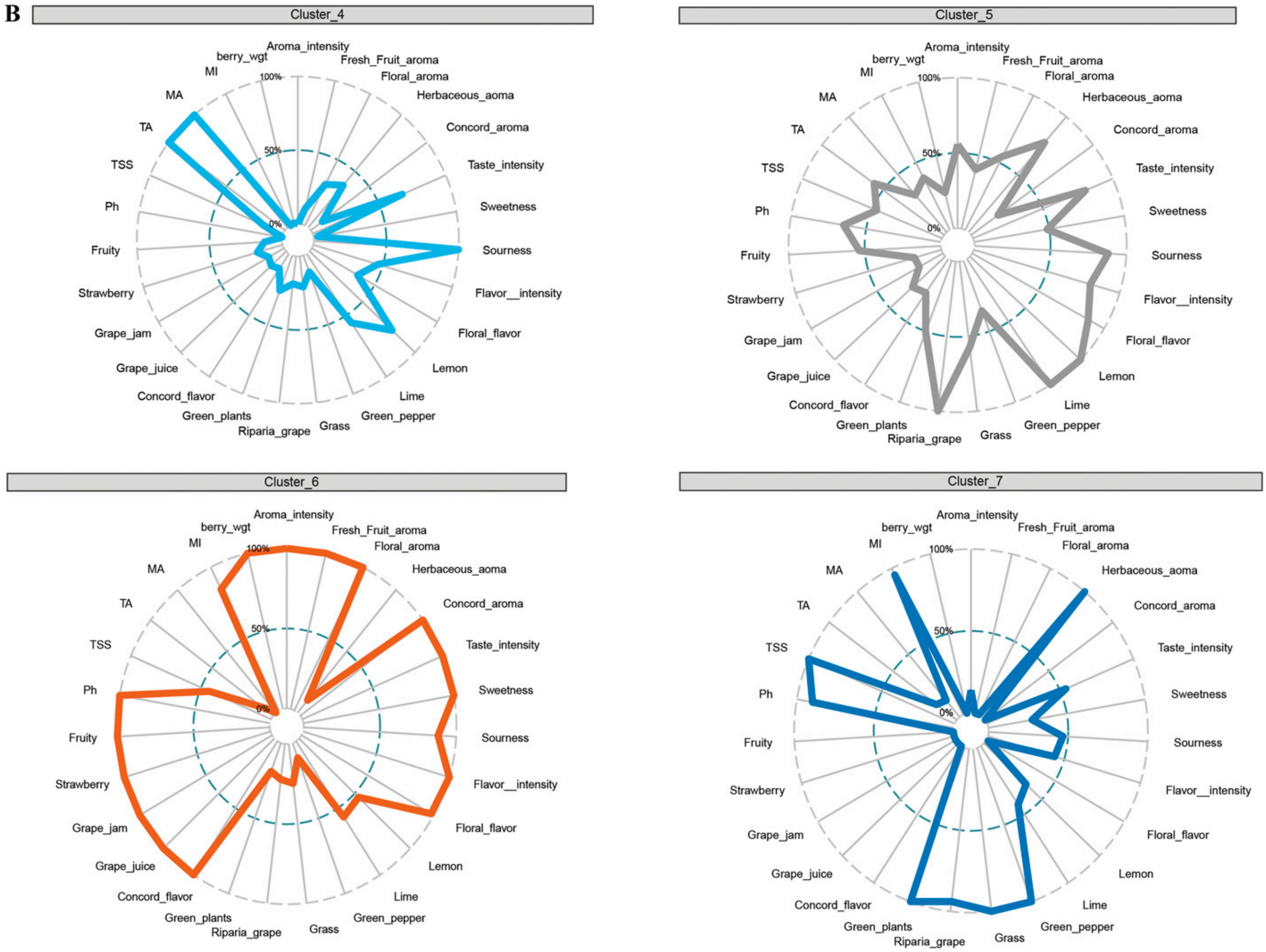

Fig. 4. (Continued)

and were grouped together. Panelists also noted high lime, lemon, sourness, and taste intensities in these clusters (Fig. 4B). However, a key distinction between these two groups was that cluster 7 had high TSS and MI.

Finally, cluster 6 (n = 5) comprised genotypes with the highest intensities of attributes associated with candy (fruity, strawberry, grape jam, Concord grape) aroma and flavor profiles compared with any other cluster characterized as labrusca type. The combination of high sweetness ratings and low TA concentrations was in contrast to the other clusters (Fig. 4B).

Characterization of GE1337 parents and grandparents

Spider plots were built using mean scores of significant sensory attributes for the population, parents, and grandparents (Fig. 5). The data suggested that flavor and aroma notes varied, and each ancestor of the GE1337 family contributed differently to the aroma and flavor profile found in the offspring. 'Itasca' and 'Frontenac' likely contributed to herbaceous flavor and aroma attributes (Fig. 5B), while MN 1250 (father) contributed to the fruity, floral, and Concord (labrusca) aroma and flavor attributes (Fig. 5C). Citrus flavors (lime and lemon) were derived from 'La Crescent' (muscat) (Fig. 5C). The grandparent MN 1198 is the second most aromatic individual based on the overall mean and is characterized by labrusca flavor and aroma attributes (Fig. 5C).

'Itasca' has high MI, TSS, herbaceous-like aroma, and flavor profiles. MN 1250 has lower TSS and higher TA than 'Itasca'. Berries from MN 1250 were rated with the highest aroma intensity score, and flavor and aroma were perceived as fresh fruit, floral, and Concord notes (Fig. 5A). 'La Crescent' and 'Frontenac gris' showed high scores for TA, MA, flavor, and taste attributes.

Discussion

The results were used to identify a subset of clusters and genotypes by prioritizing those with desirable sensory profiles, such as high intensities of fruity, floral, and labrusca (grape juice, Concord, and grape jam) flavors while minimizing undesirable herbaceous and green flavors. A cluster analysis identified clusters with these preferred profiles that could be targeted within the breeding program. The correlation among sensory attributes and chemical metrics (TSS, TA, and MI) was used to identify clusters with different levels of sweetness and acidity, which are essential for consumer preference.

A year effect was detected in this study that could be attributed to environmental factors but also differences of sample availability each year and different, although trained, panelists. Differences in temperature and other weather conditions, especially during critical stages of vine growth and berry ripening, likely influenced the development of sensory attributes (Supplemental Table 4). Such environmental variability can explain the statistically significant year effect observed for the overall intensities of aroma, flavor, and taste, as well as for specific attributes like floral aroma, lemon, lime, citronella, fruity, and herbaceous flavors.

Panelists effects contributed significantly to the variation in nearly all attributes, as anticipated in this incomplete block design where different panelists evaluated distinct sets of samples. This aligns with the common observation of a notable impact of panelists on descriptive analysis studies, even in complete block designs where all panelists assess identical samples (Gardner et al. 2017; López et al. 2011; López-López et al. 2019; Savits

A

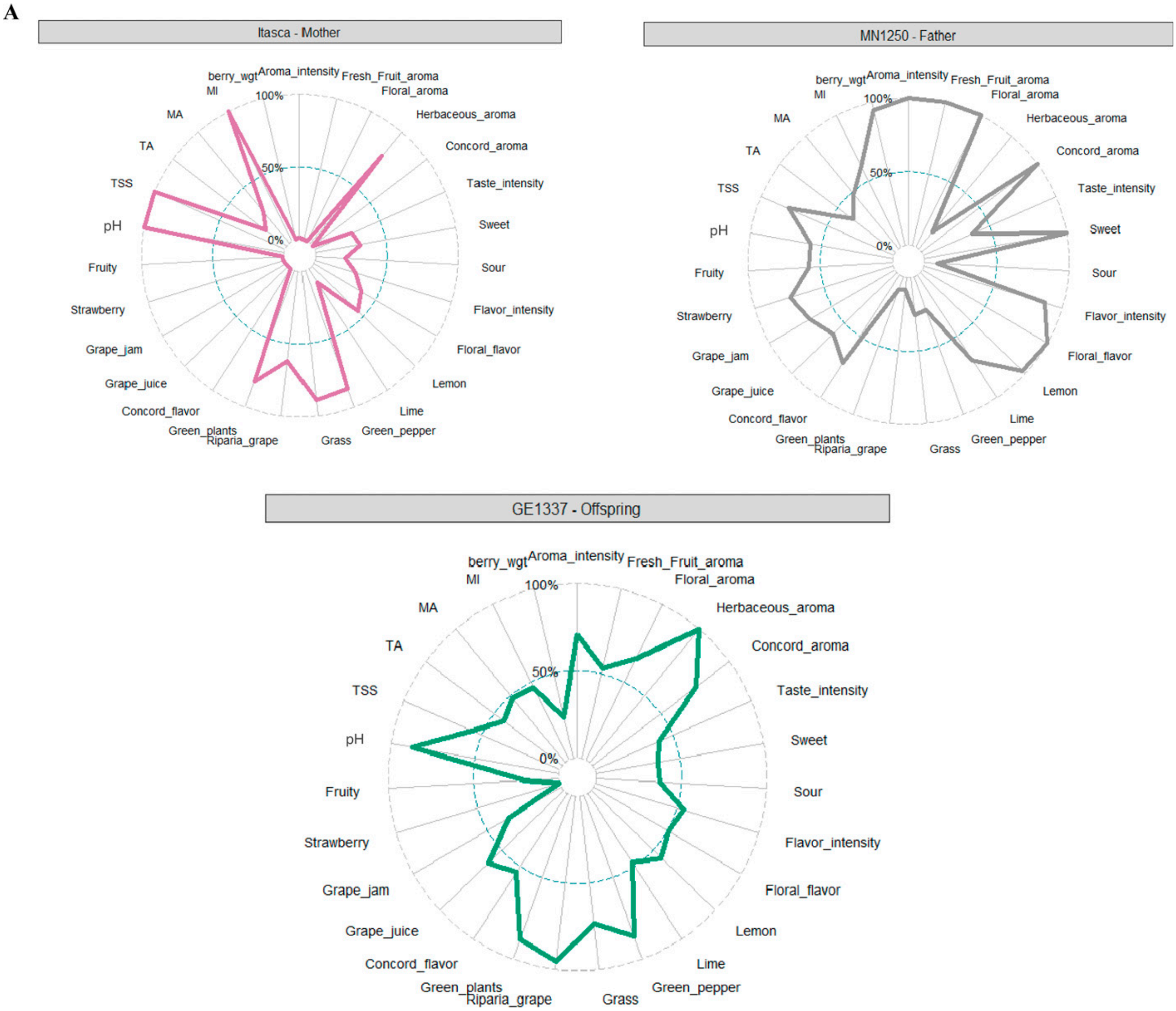

Fig. 5. Spider diagrams illustrating the difference in significant sensory attributes based on a three-way analysis of variance (ANOVA) ($P < 0.05$) of berries assessed from (**A**) parents ('Itasca' and MN1250) and GE1337 offspring, (**B**) maternal grandparents ('Frontenac gris' and MN1234), and (**C**) paternal grandparents ('La Crescent' and MN1198).

2014). The significant effect of the panelist was likely explained by how panelists used the intensity line scale differently (Douglas et al. 2001; Hakimi Rezaei and Reynolds 2010; López-López et al. 2019; Meilgaard et al. 1999; Savits 2014).

The use of control samples and their clustering with the 'Itasca' sample supported the effectiveness of this methodology. When conducting sensory studies with a balanced incomplete block design to assess a large number of samples, we recommend incorporating a standard sample or samples for the purpose of validating data quality and evaluating how panelists rate all attributes included in the survey.

Panelists observed that the intensity of the green plant attribute was equivalent to the other four attributes (green pepper, grass, riparia grape, green apple) within the herbaceous flavor category (Fig. 4). This suggested that panelists were either unable to delineate or unable to detect differences within this herbaceous category, suggesting they needed additional training or that the lexicon was too complex. Chambers and Koppel (2013) affirmed that clear definitions and proper training of sensory panels could also reduce misunderstanding by using different terms to describe the same sensory phenomenon. Likewise, certain key attributes require more training to measure the nuances in aroma differences among samples.

Correlation among basic chemistry properties and sensory perception

In this study, the correlation between fundamental chemistry properties and sensory perception was not consistent with that reported by previous studies. Authors who studied table grapes have observed a lack of correlations among TSS, TA, and sensory attributes (Jayasena and Cameron 2008; Maoz et al. 2020). Grape samples in this study with high TA and MA were perceived as sour and as having herbaceous flavor and aroma. Additionally, MA can significantly influence the sensory properties of grapes and wines, increasing the sensory perceptions of tartness, astringency, and herbaceous or "grassy" flavors (Kallithraka et al. 1997). Sour grapes may affect the eating experience, damage a new variety's reputation, and cause problems in wines and the vinification process. Typically, tartaric acid and malic acid account for 90% of the acids observed in grapes, with tartaric acid found in higher quantities (Lamikanra et al. 1995).

The correlation results demonstrated that grape samples with high TSS and MI were perceived as sweet and having floral and fruity or labrusca flavor and aroma attributes. As a result of the growing understanding of interactions of taste × retronasal olfaction, it was not unexpected that the correlations between TSS and fruity contributed to the

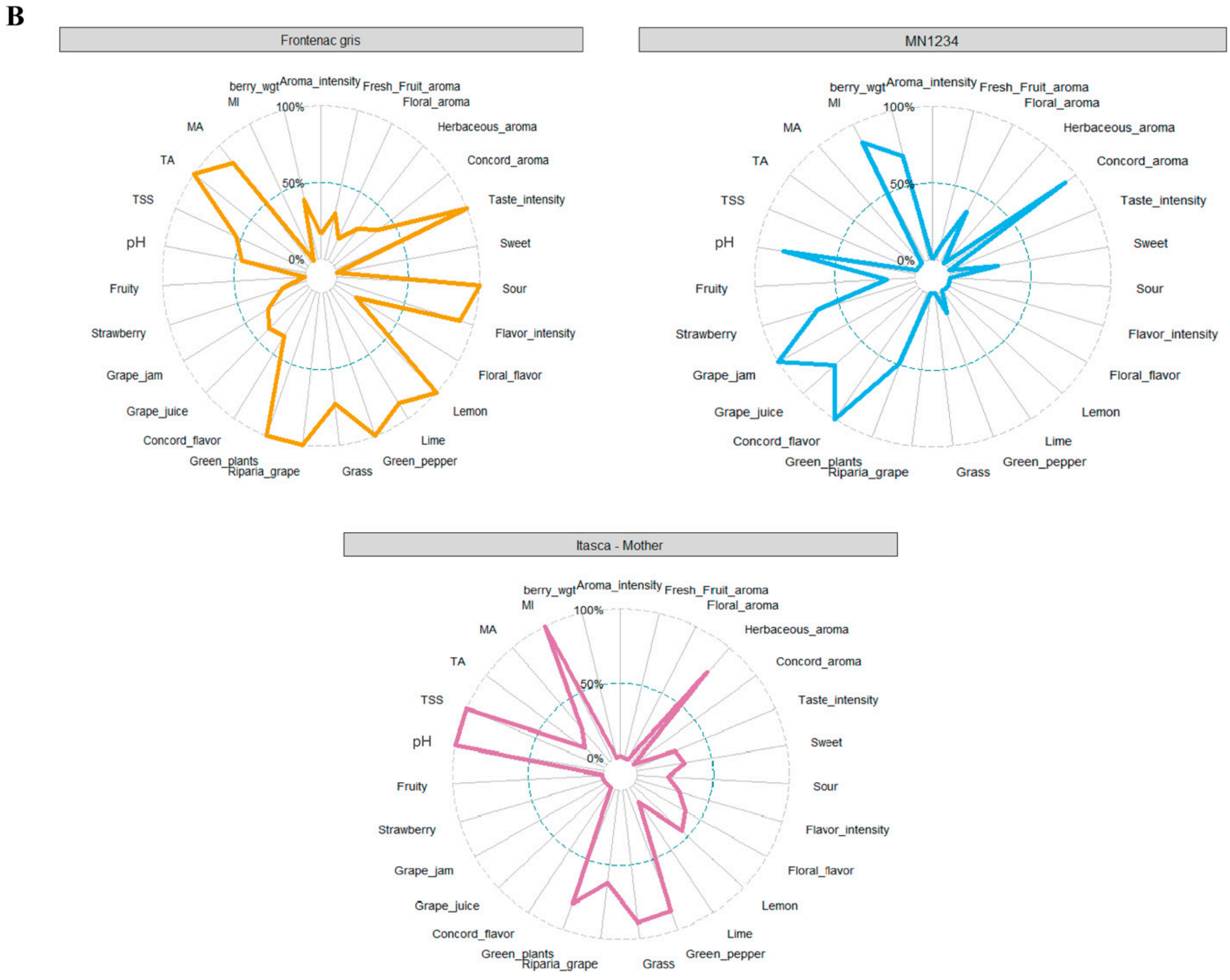

Fig. 5. (Continued)

perceived sweetness of grapes. The TSS plays a significant role in the perceived sweetness of grapes because higher TSS levels correspond to increased sweetness perception by sensory evaluators (Maoz et al. 2020).

Berries from ‘Frontenac’ and ‘Frontenac gris’ were rated with high intensities of fruity and jammy attributes as the sweetness of the grapes increased (Brady 2017; Del Bel 2014). Panelists confused the sweetness sensation with flavor perception (Maoz et al. 2020); sweetness (a sensation on the tongue) cannot be smelled. However, aroma perceptions have been found to increase the perceptions of sweetness (Bertelsen et al. 2020).

Among all sensory attributes and the chemistry metrics investigated in this study, TSS and sweetness are the predominant drivers of consumer preferences for fresh grapes (Jayasena and Cameron 2008; Uddin et al. 2023), and many descriptive analysis studies have shown that TSS increases the overall level of likeability of a sample (Maoz et al. 2020). A similar pattern in apples was also reported (Hampson et al. 2000).

Flavor and aroma characterization of the GE1337 population

Cluster 1 grouped genotypes with neutral flavor and aroma profiles. Neutral flavor is defined by the lack of aromatic compounds and is perceived as having a low flavor intensity. In grapes, neutral flavor is attributed to grape berries lacking monoterpenes (Mateo and Jiménez 2000). Neutral grapes in the University of Minnesota breeding program may be suitable for wine grapes because they lack the aromas typical of *V. labrusca* or *V. riparia*.

Fruity flavor is difficult to define, and the compounds associated with this flavor attribute depend on the fruit crop. For grapes, optimal harvest time and fruity flavor have been associated with monoterpene and ester compounds (Maoz et al. 2020; Wang and De Luca 2005).

Grapes are considered muscat-flavored when the level of monoterpenes reaches 6 $\mu g \cdot g^{-1}$ (Mateo and Jiménez 2000), and the floral flavor is the predominant perceived flavor. Concord grape flavor (*V. labrusca*) is widely used to describe the classic grape flavor of that species. Methyl anthranilate is one of the main compounds driving the cultivar flavor. Methyl anthranilate is also used as an artificial strawberry flavor and the main natural component giving character to the fragrance of the very popular Welch’s grape juice (Maoz et al. 2020; Wang and De Luca 2005). Green or herbaceous attributes are characterized by either the presence of methoxypyrazines, C6 aldehydes, or alcohols.

Clusters 2 and 6 exhibited high intensities of fruity, Concord, and floral flavors. Individuals with a combination of herbaceous and labrusca were not observed in this study, indicating that these two categories are likely antagonists and further supported by the negative correlation observed in the results. Further investigation may be necessary to reveal the genetic foundation for this association. Individuals within cluster 6 were described by panelists using attributes such as high sweetness ratings and low TA concentrations. This distinctive combination, in contrast to the other clusters, may explain the choice of these attributes to characterize individuals in this specific group.

Fig. 5. (Continued)

Characterization of parents and grandparents of the GE1337 family

Despite exhibiting high levels of TSS and MI, 'Itasca' was characterized by herbaceous aroma and flavor, with notes of green pepper and green plants. Clark et al. (2017) described the flavor in 'Itasca' wine as pear, quince, star fruit, melon, and subtle honey notes; these attributes were different from those identified in this study. Interestingly, herbaceous was not listed as one of the flavors in wine made of 'Itasca', but there are plausible biological, viticultural, and marketing explanations. Regarding the biological explanation, Clark et al. (2017) described 'Itasca' flavor based on berries with high TSS (<24 °Brix), which could contribute to the expression of the reported flavor profiles. A high TSS content combined with different wine-making techniques might promote the expression of fruity and honey flavor profiles. The lexicon developed in the current study had not yet been developed and could be used for future cultivar release papers. Regarding the viticultural explanation, fruit reported previously had been harvested at a more mature stage. Regarding the marketing strategy, the herbaceous attribute detected in low abundance was not listed in the cultivar release paper because it is not an appealing term to describe the flavor of a new cultivar. Traditionally, wines made from hybrid grapes with a *V. riparia* species in the pedigree have a poor reputation because of the presence of off-flavors described as grassy, green, vegetal, or herbaceous (Boulton et al. 1986; Haggerty 2013; Lei et al. 2018).

In this study, sensory attributes primarily identified in 'Frontenac gris' included green plants, riparia, and grassy notes. The herbaceous and green plant aroma and flavor have been reported for fresh berries and wines of 'Frontenac gris' (Brady 2017; Mansfield and Vickers 2009). These aromas are commonly associated with *V. riparia* (Brady 2017). Unfortunately, often black (red berried) hybrid grapes can be a source of green aroma and flavors generally undesirable in red wine (Kemp et al. 2019); however, at low levels, they are signatures of cultivar such as Cabernet Sauvignon and Sauvignon blanc (Lei et al. 2018).

The panelists detected intense lemon and lime flavors in 'La Crescent', which may be associated with the aroma × taste interaction with TA × MA contents present in the berries. 'La Crescent' is considered a muscat cultivar with high terpenes and lemon flavor. Peach and apricot have been reported as flavors in wines made from 'La Crescent' grapes (Savits 2014). However, in our study, panelists did not perceive peach and apricot flavor attributes in the berries, indicating that flavor and aroma could also be masked by the berry (under)-ripeness or is a product of the wine-making technique used by many wineries. Grass flavor and herbaceous aroma were detected in 'La Crescent' as well, but these flavors and aromas have not been reported for 'La Crescent' wines (Mansfield and Vickers 2009; Ruiz et al. 2019).

This study has provided a new framework for investigating the sensory properties of fresh grapes, wines, and other fruit crops.

By examining the development of a lexicon and evaluating parents and grandparents, this experiment has captured the variation in the population for multiple flavor attributes and is a significant improvement over previous studies that focused on families segregating for only one flavor (Battilana et al. 2009; Doligez et al. 2006; Emanuelli et al. 2010; Ruiz-García et al. 2014; Wu et al. 2013). The methodology introduced in this study was able to collect high-quality data for further genetic studies and select individuals with fruity/floral flavor attributes to be used in table grape breeding as well individuals with a lack of labrusca or herbaceous flavor attributes to be used as parents in wine breeding activities. Therefore, the present methodology may enhance breeding programs by facilitating data collection for genetic studies or identification and selection of attributes that contribute to desirable flavor and aroma profiles in fruit crops.

Conclusion

The family used in this study segregated for different aroma and flavor attributes. The most promising individuals for table grape breeding were aggregated in cluster 6 and characterized by fruity, floral, and labrusca (Concord) attributes. Fruity flavor has already been reported as correlating with berry preference. Additionally, genotypes in these clusters did not show a green or herbaceous aroma and flavor, which are attributes not appreciated by consumers. Herbaceous attributes are associated with some key cultivars such as Cabernet Sauvignon, but overabundance of these compounds, especially in crop wild relatives and progenitors for cold hardiness, are harsh and excessive. Thus, these seedlings may be more useful germplasm for table grape breeding objectives. Flavor and aroma from genotypes in clusters 1 and 2 were perceived as neutral, which also are potential targets for wine grape breeding for nonmuscat wines without negative hybrid characteristics.

We recommend that fruit breeders should implement our experimental design framework to gain a better understanding of their populations for sensory properties of mapping family populations, parents, and advanced selections in food crops, especially fruit.

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