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Intra-plot, and intra-plant variability in a vineyard of Catarratto Lucido (*Vitis vinifera* L.) in Sicily

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Abstract. Understanding intra-plot to intra-plantvariability is essential for optimizing grape maturity monitoring, harvest time, and sampling protocols. This study investigates the importance of intra-plant variability in grapevine sampling and evaluates whether it should be integrated into protocols to enhance accuracy and improve vineyard management. The study was conducted in a commercial vineyard in the Alcamo PDO area, Sicily, using 24 Catarratto Lucido vines. These vines were categorized into high-vigor (HV) and low-vigor (LV) groups based on Normalized Difference Vegetation Index (NDVI) analysis. Intra-plant variability was assessed for key grape characteristics, including Total Soluble Solids (TSS), pH, acidity, and bunch weight, across different spur, node, and bunch positions. Overall, node position significantly reduced bunch acidity ($\beta=-0.310$; $p=0.0382$), spur position negatively impacted TSS ($\beta=-0.78$; $p=0.0317$), and bunch weight was significantly higher on basal bunches (A) than apical ones (B)($\beta=-65.642$; $p<0.0001$). Conversely, no significant positional effects were identified in LV vines. Our findings indicate limited intra-plant variability, but these observations require confirmation through multi-year studies including climatic and physiological parameters.

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1. Introduction

In viticulture, understanding variability at multiple scales is essential for effective vineyard management, including monitoring grape maturity, determining optimal harvest dates, and optimizing sampling protocols. On the broader-levelintra-vineyard variability may be induced by topographical, pedological factorsand even plant materials and training managements. This variability may significantly affect grape maturity and composition, hence winemaking decisions. Nowadays remote sensing tools, such as the Normalized Difference Vegetation Index (NDVI), have improved the ability to identify and manage vineyard variability by offering precise insights into spatial heterogeneity [1]**.** However, these tools are generally unable to detect intra-plant variability in terms of Total Soluble Solids (TSS), pH, and titratable acidity.Such variation grapevines can arise from factors such as berry-to-berry differences within a bunch, bunch and shoot position, fruit set timing, shoot diameter, genetic and molecular differences, and acrotony (apical dominance), a common issue in the Guyot pruning system, leading to uneven grape maturation [2,3].Over the last 50 years, several sampling protocols have been developed toaddress variability and optimize sampling protocols. For instance, [4]proposed cluster sampling from 20 rows with five bunches per row. [5]recommended sampling either two sets of 50 berries or five sets of 10 bunches per block, depending on vineyard heterogeneity. [6]suggested collecting basal clusters from shoots located at equal distance between the trunk and the tip of the fruiting branch. Despite advancements in plot-level sampling protocols, intra-plant variabilitydifferences in grape characteristics across nodes, spurs, and bunch positions within a single vineremainsunderexploredpoorly understood [7]**.**Therefore, in case of intra-plant variability such random sampling methods commonly used in vineyard management may fail to capture differences leading to biased or incomplete data.This study investigates the importance of intra-plant variability in grapevine sampling by analyzing differences in TSS, pH, bunch weight, and acidity across different nodes, spurs, and bunch positions in two different vigor level:high-vigor (HV) and low-vigor (LV). We aim to determine whether intra-plant variability should be integrated into sampling protocols to enhance accuracy, capture variability, and improve vineyard management decisions.

2. Materials and Methods

2.1. Study Area

The study was conducted in 2023 in a commercial vineyard domain within the Alcamo Protected Designation of Origin (PDO) area, located in the hinterland of western Sicily (Italy) at Tenuta Rapitalàwinery (37°55′9.61″N; 13°4′28.59″E). The vineyard, situated at an altitude of 315m.a.s.l, experiences a typical Mediterranean climate with an average annual rainfall of approximately 558 mm (SIAS). The vineyard is drip irrigated, and the vines are trained on a VPS shoot positioning system, double cordon spur pruned, planted with a spacing of 2.40 m × 0.9 m between and within rows.

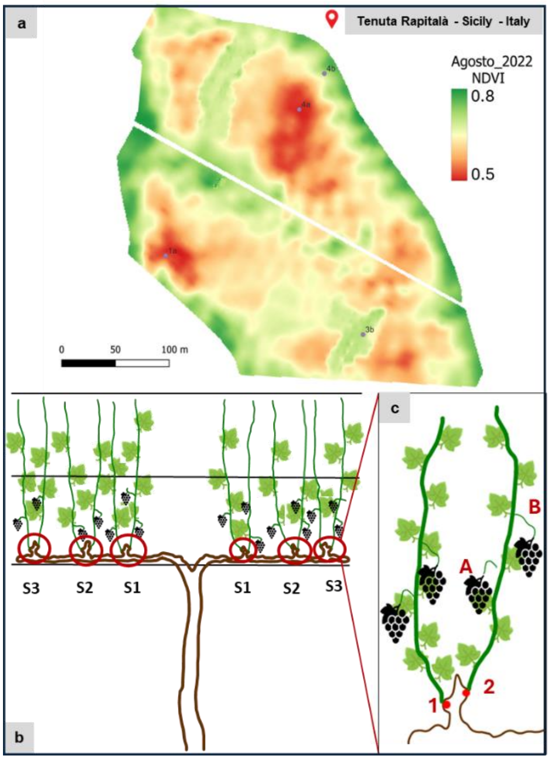

Figure 1. a) Study area map with the NDVI scale of the studied four plot [High vigor (HV) indicated as 4a and 1a on the map; low vigor (LV), indicated as 4b and 3b] b) Spurs position (S1, S2, S3) repartition across the vine. c) node position (N1, N2), and bunch position on each node (A, B).

2.2. Plant material and analysis

From this domain a total of 24 vines of the cultivar Catarratto Lucido (*Vitis vinifera* L.), grafted onto 1103P rootstockhas been used for the field experiment. were used for the experiment.Four plots were selected, comprising two high-vigor (HV) and two low-vigor (LV) plots, with six vines per plot, and a total 12 vines per vigorclass. Plot vigor was classified based on the pruning wood weight measured in January 2022. This classification was then validated throughNDVI ranges (LV: 0.4-0.55; HV; 0.6-0.9)derived from remotesensing analysis acquired during August 2022with a flying drone (DJI)(Figure 1a). The vigor classes werethen validated and confirmed through measuring pruning wood weight later this year.To assess intra-plant variability, grape bunches were categorized by their position on the vine within each vigor zone, considering spur position (S1, S2, S3), node position (N1, N2), and bunch position(A, B) (Figure1b,c). Variability of the bunch position was only considered when two bunches were present on the same node. At the harvest time (BBCH 89) all available bunch on the vine were collected and labeled as *[Vigor (LV-HV), plant number (1-12), spur position (1-2-3), node position (1-2), bunch position (A-B)]*. Each bunch separately was then assessed for weight (g), total soluble solids (TSS, °Brix) using an optical refractometer (HANNA Instruments Italia srl), pH, and titratable acidity (g/L of tartaric acid) analyzed with an automatic titrator (Crison Instruments SA, Barcelona, Spain).

2.3. Meteorological Data

Meteorological data including daily maximum temperature (T max °C), daily minimum temperature (T min, °C), daily average temperature (T avg, °C) and daily rainfall (mm) for the year 2023 were retrieved from a local meteorological station installed in the vineyard.

2.4. Data Analysis

The relationship between bunch characteristics and the measured dependent variables was analyzed using General Linear Models (GLMs). Each dependent variable (bunch weight, acidity, pH, and TSS) was modeled using the following independent variables and their interactions: vigor (HV, LV), spur position (S1, S2, S3), node position (N1,N2), and bunch position (A,B). Statistical significance was set at $p<0.05$. Then, within each vigor level and to further investigate intra-plant variability, separate MANOVA tests to assess the combined effects of node, spur, and bunch position on the dependent variables (bunch weight, pH, titratable acidity, and TSS). Post-hoc univariate ANOVAs were used to identify significant effects on individual dependent variables where applicable. All analyses were performed in R (version 4.2.3).

3. Result

The meteorological data recorded an annual average daily temperature of 17.8 °C, with a highest peak of 43 °C in July and a lowest peak of 2 °C in January. The total annual rainfall was 315 mm (Figure 2).At harvest, an average of six bunches per vine in LV plants and nine bunches per vine in HV plants were harvested and recorded. Bunch weight averaged 138.5 g with no differences between LV and HV. An average of 5.2 g/L was measured in HV plants, compared to 4.7 in LV plants (p-adj < 0.0006) (Figure 3). No statistical differences were observed in TSS or pH, which averaged 20.5 °Brix and 4.5, respectively, across both vigor levels. ThroughGLMs analysis we identified a few significant intra-plant variability and effect of bunch positioning on certain bunch characteristics. The node N2 had a significant negative effect on the acidity level of bunches($\beta=-0.310$; $p = 0.0382$). As to Spurs, S3 showed a significant negative association with the TSS level ($\beta=-0.78$; $p=0.0317$). Bunch weight was found significantly lower for bunch position B compared to position A ($\beta=-65.642$; $p<0.0001$). Overall, GLMs did not show significant effects on any interaction across bunch positioning and measured variables across vigor levels (Table 1).On each vigor level separately and through MANOVA, we observed distinct patterns of intra-plant variability between HV and LV vines. In HV vines, bunch position had a significant impact on bunch weight (MANOVA, $p<0.05$; ANOVA, $p<0.001$) bunch A were larger bunches than bunch B. On the other hand, bunch observed on N2 had higher TSS, than those gathered on N1 ($p=0.033$). Spur positions had no significant effects ($p>0.05$) in HV vines. For LV vines, no significant influenced of the node, spur, or bunch position ($p> 0.05$) were observed (Table 1).

Table 1.table shows only significant effects and interactions from GLMs, MANOVA and ANOVA. Node 1, Spurs 1 and bunch A were considered as baseline reference in the GLM.

General Linear Models					
Dependent Variable	**Term**	**Estimate**	**Std. E**	**t value**	**P**
Acidity	Node 2	-0.30	0.15	-2.09	0.03
TSS	Spurs 3	-0.78	0.36	-2.17	0.03
Bunch weight	Bunch B	-65.64	14.99	-4.37	0.00
MANOVA (HV)					
Effect	**Df**	**Appro F**	**NumDf**	**DenDf**	**Pr(>F)**
Bunch Pos	1	5.8950	4	86	0.000
Post-Hoc Anova (HV)					
Dependent Variable	**Effect**	**Df**	**Mean Sq**	**F**	**P**
Bunch Weight	Bunch Pos	1	73784	21	1.4e-05
Acidity	Node	1	2.23	4.07	0.03

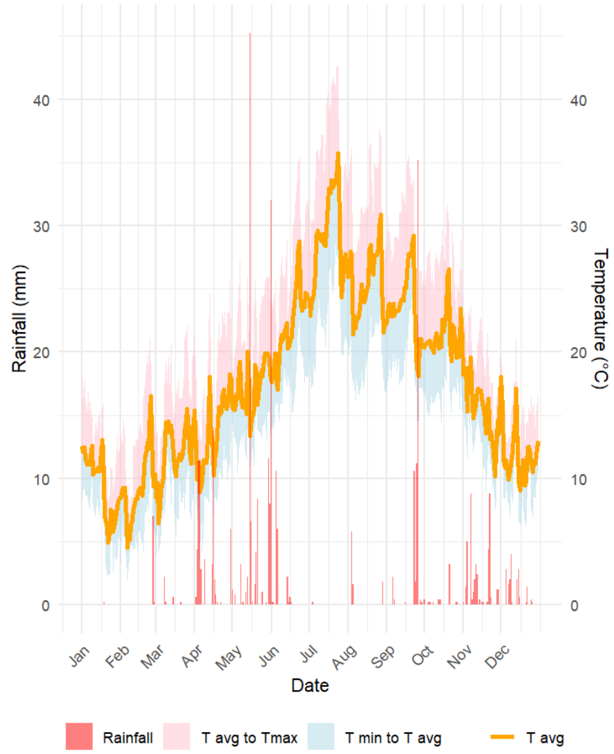

Figure 2. A graph presenting the daily average temperature (T avg, orange line), along with the daily minimum temperature (T min, sky-blue shaded area) and the daily maximum temperature (T max, light pink shaded area) ispresented alongside daily rainfall (red bars) recorded across the vineyard for the year 2023.

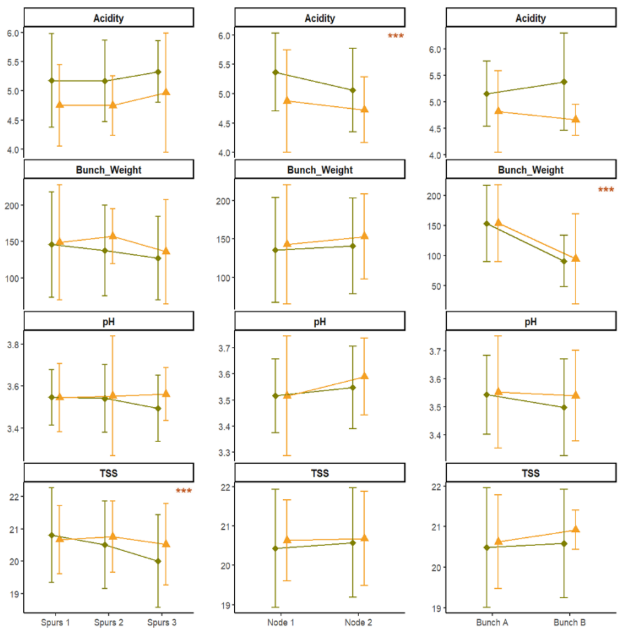

Figure 3. Comparison of bunch weight acidity, pH, and TSS across spur, node, and bunch positions in high vigor (HV, green line) and low-vigor (LV, orange) plants. Significant differences obtained by the GLM are highlighted with ***. Error bars represent the standard deviations (Std E).

4. Discussion

It is well-established that within a single vine, not all bunches exhibit the same degree of ripeness. A recent study **[8]** even supported the idea of multiple day harvesting to ensure better quality of grape**[11]**. However, it remains unclear whether this variability can fail to be observed through commonly adopted randomized sampling protocols. To optimize these protocols, it is needed to determine whether incorporating intra-plant variability is necessary for future methodologies.When, examining intra-vineyard variability across the two studied vigor levels (low and high vigor), no significant differences were detected in grape composition (TSS, pH, or grape weight). One limitation of these results could be the influence of seasonal climatic conditions, as variations in temperature and rainfallpatterns may have either emphasized or mitigated differences between vigor levels.This was evident in the findings of **[9]**, wherecontrasting results were observed over the yeardue to the strong seasonal effects when comparing several grape parameters across low and high-vigor vineyard.At the intra-plant level, our results revealed significant differences. For spur positions, a negative association was observed between the third spur (S3) and TSS levels. Similar findings were reported by **[10]**, who noted inconsistent results in a bilateral cane training system. In the second year of their study, apical shoots had higher TSS levels than mid-cane and proximal shoots, aligning with our findings, while no differences were observed in the first year. Conversely, **[6]** found that spurs farther from the trunk exhibited higher TSS content. Overall, bunch position variability was evident only in high-vigor vines, with no significant differences noted in low-vigor vines. Our findings showed that bunch weight was significantly lower for position B compared to position A, particularly in high-vigor vines. Our results align with which reported a higher basal bunch weights compared to apical ones on the same shoot was. Such variability is not limited to investigated chemical parameters; bunch position was also found to influence phenolic and aromatic content, which can significantly impact wine quality Despite the effect of bunch position on weight, TSS, acidity, and pH did not vary significantly between bunchesdocumented similar results, where only 3 of 14 cultivars showed significant TSS variability based on bunch position. Interestingly, acidity displayed an opposite trend, with higher titratable acidity (TA) in the fourth bunch compared to the first. also reported varying results for TSS, TA, and pH based on bunch position over two years. In their first year, basal bunches had significantly higher TSS levels than apical ones, while no differences were observed for pH and acidity. In the second year, apical bunches exhibited higher TA and pH levelsnoted that acidity wasnot influenced by bunch position, but lower bunches tended to have higher TSS levels than upper ones.Variability in grape composition has been demonstrated in several studies. This is generally attributed to the earlier burst of distal buds compared to basal or intermediate buds, resulting in earlier formation and emergence of inflorescences. Consequently, when comparing grape composition at the same time, distal bunches may have reached maturity earlier than basal or intermediate bunches. This variability has also been linked to shoot diameter. **[11]** proposed that shoots with larger diameters are more likely to develop inflorescences with an outer arm (wing), which tends to mature later.In addition to physiological factors, contrasting results across multi-year studies on grape composition variability have been linked to inter-annual climatic differences, such as temperature and rainfall **[12]**. These factorslikely contribute significantly to year-to-year variations in bunch composition. In our study, total annual rainfall in 2023 amounted to 356.4 mm, which is significantly lower than the cumulative average monthly rainfall over the past 20 years (850.01 mm, based on SIAS – ServizioInformativoAgrometeorologicoSiciliano, ww.sias.regione.sicilia.it). This discrepancy may have influenced our findings.

5. Conclusion

Intra-plant variability appears to have limited influence on acidity, TSS levels, and bunch weight, primarily influenced by bunch position on the shoot, spur, and node position.However, such variation cannot be entirely ruled out, especially given the limitations of single-year data. In this context we suggest a multi-year study to confirm these findings, accounting for seasonal variations and factors such as intra-bunch differences, abiotic influences, and physiological parameters like water stress and photosynthesis. We recommend developing standardized sampling protocols tailored to specific cultivars, regions, or seasons rather than relying on a universal model.

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