Identification of SNPs associated with magnesium and sodium uptake and the effect of their accumulation on micro and macro nutrient levels in *Vitis vinifera*

Rachel P Naegele [Corresp., 1], **Jason P. Londo** [2], **Peter Cousins** [3]

[1] San Joaquin Valley Agricultural Sciences Center, United State Department of Agriculture, Parlier, California, United States

[2] Grape Genetics Unit, United State Department of Agriculture, Geneva, New York, United States

[3] E & J Gallo Wineries, Modesto, California, United States

Corresponding Author: Rachel P Naegele
Email address: rachel.naegele@usda.gov

Macro and micro nutrient accumulation affects all stages of plant growth and development. When nutrient deficiencies or excesses occur, normal plant growth is altered resulting in symptoms such as leaf chlorosis, plant stunting or death. In grapes, few genomic regions associated with nutrient accumulation or deficiencies have been identified or studied. Our study evaluated micro and macro nutrient concentrations in *Vitis* to identify associated SNPs. Nutrient concentrations and foliar symptoms (leaf chlorosis and stunting) were compared among 249 F_1 *Vitis vinifera* individuals in 2015 and 2016. Foliar symptoms were consistent (≥ 90%) between years and correlated with changes in nutrient concentrations of magnesium ($r = 0.65$ and $r = 0.38$ in 2015 and 2016, respectively), aluminum ($r = 0.24$ and $r = 0.49$), iron ($r = 0.21$ and $r = 0.49$), and sodium ($r = 0.32$ and $r = 0.21$). Single nucleotide polymorphisms associated with symptoms (2015 and 2016), sodium (2015 and 2016), and magnesium (2015) were detected on chromosomes 3, 10, 11, 12, 13, 14, 18. Symptoms and magnesium concentration were primarily associated with SNPs on chromosome 3, while sodium-associated SNPs were found on chromosomes 11, 12, 13, and 18. Mean concentrations for each nutrient varied between years in the population between symptomatic and asymptomatic plants, but relative relationships were mostly consistent. These data suggest a complex relationship among foliar symptoms and micro and macro nutrients accumulating in grapevines.

Identification of SNPs associated with magnesium and sodium uptake and the effect of their accumulation on micro and macro nutrient levels in *Vitis vinifera*

Rachel P. Naegele[1], Jason P. Londo[2] and Peter Cousins[3]

[1] USDA ARS San Joaquin Valley Agricultural Sciences Center Parlier, CA 93648
[2] USDA ARS Grape Genetics Unit Geneva, NY
[3] E&J Gallo Wineries Modesto, CA

Corresponding Author:
Rachel Naegele
9611 S. Riverbend Ave Parlier, CA 93648
Email address: Rachel.naegele@usda.gov

Abstract

Macro and micro nutrient accumulation affects all stages of plant growth and development. When nutrient deficiencies or excesses occur, normal plant growth is altered resulting in symptoms such as leaf chlorosis, plant stunting or death. In grapes, few genomic regions associated with nutrient accumulation or deficiencies have been identified. Our study evaluated micro and macro nutrient concentrations in *Vitis* to identify associated SNPs. Nutrient concentrations and foliar symptoms (leaf chlorosis and stunting) were compared among 249 F_1 *Vitis vinifera* individuals in 2015 and 2016. Foliar symptoms were consistent (≥ 90%) between years and correlated with changes in nutrient concentrations of magnesium ($r = 0.65$ and $r = 0.38$ in 2015 and 2016, respectively), aluminum ($r = 0.24$ and $r = 0.49$), iron ($r = 0.21$ and $r = 0.49$), and sodium ($r = 0.32$ and $r = 0.21$). Single nucleotide polymorphisms associated with symptoms, sodium, and magnesium were detected on each chromosome with the exception of 5 and 7 depending on the trait and genome used for analyses. Symptoms and magnesium concentration were primarily associated with SNPs on chromosome 3, while sodium-associated SNPs were primarily found on chromosome 11. Mean concentrations for each nutrient varied between years in the population between symptomatic and asymptomatic plants, but relative relationships were mostly consistent. These data suggest a complex relationship among foliar symptoms and micro and macro nutrients accumulating in grapevines.

Introduction

Macro and micronutrients are essential for proper cell function and overall plant health. Macronutrients, those needed in large quantities by plants, include nitrogen, phosphorus, potassium, calcium, sulfur, and magnesium. These are largely present in the soil and are readily available to plants depending on soil pH and moisture (Maathuis 2009). Micronutrients, such as sodium, boron, iron, zinc, manganese and copper, are less prevalent in the soil, but small quantities are still necessary for plant growth and development. Nutrient levels fluctuate in the plant, and vary based on developmental stage, maturity, genotype, and tissue (Benito *et al*. 2013; Pradubsuk and Davenport 2010). In grape, a perennial woody vine, nutrient fluctuations occur throughout the season with specific nutrient concentrations peaking during critical periods of development and growth. The composition and quantities of these nutrients can have drastic effects on fruit quality and plant health pre and postharvest (Conradie 1981; Conradie 1992; Morris *et al*. 1983; Mpelasoka *et al*. 2003; Rogiers *et al*. 2000; Schreiner 2016; Williams *et al*. 2004).

Nutrient deficiencies often result from poor ion availability or uptake, leading to deformation of shoots or roots, uneven ripening of fruit, and chlorosis or necrosis of leaves. Leaf chlorosis is a common symptom of nutrient deficiency, as many macro and micronutrients contribute to chlorophyll production, enzyme and membrane stabilization and activation. Magnesium (Mg) is an important structural component of chlorophyll and a phosphorylizer or dephosphorilizer of compounds. Symptoms of Mg deficiency, such as interveinal chlorosis of the leaves, necrotic leaf spots, and root and shoot stunting can be induced by low levels of Mg or high levels of calcium (Ca), potassium (K) or other ions, which can alter Mg absorption (Guo *et al.* 2016; Hermans and Verbruggen 2005; Skinner and Matthews 1990; Spiers and Braswell 1994).

Sodium (Na) can be used by plants in small quantities, but in excess, causes stunting, leaf tip burning, and leaf darkening (Bernstein 1975). Leaf chlorosis, found in many nutrient deficiencies, is not a characteristic symptom of Na excess, except as a result of cation imbalances. These imbalances can be the result of substrate competition, as is the case with Mg, K and Ca, or can occur through changes in ion potential and turgor pressure (Grattan and Grieve

1992; Zhu *et al.* 1998). This complex relationship, while not well studied, varies among host species and type of salt ions (Carbonell-Barrachina *et al.* 2008; Cordovilla *et al.* 2008; Volkmar *et al.* 1998). Complex relationships are also true among metal ions and nutrients in the soil. Aluminum (Al), a highly abundant metal in earth's crust, is one of the major factors limiting crop production in low pH soils (Mossor-Petraszewska 2001). Aluminum competes with other ions such as Mg or Ca for binding sites in the plant, leading to root deformation and nutrient deficiencies. It is often the lack of essential nutrients, and not the accumulation of toxic metals, that results in metal toxicity symptoms.

In *Vitis vinifera*, nutrient deficiencies are commonly observed in poor quality soils and can affect bud development, fruit yield, and quality (Brancadoro *et al.* 1995; Sinilal *et al.* 2011; Tagliavini and Rombola 2001). Fe and Mg are two of the most common deficiencies observed in grape, often observed as interveinal chlorosis (Brancadoro *et al.* 1995; Conradie and Saayman 1989). Common nutrient excesses include Na and K (Downtown 1977; Gong *et al.* 2015). However, the severity of response can vary greatly depending on the genotype used and level of excess (Kocsis and Walker 2003; Porro *et al.* 2013).

Genotypic variation in nutrient levels is often caused by differences in the ability of a plant to uptake, accumulate, or metabolize nutrients (Christensen 1984). Studies on the genetic control of nutrient accumulation in grape are limited, those that exist merely show the complexity surrounding nutrient absorption and their interactions (Davies *et al.* 2006; Jimenez *et al.* 2007; Perez-Castro *et al.* 2012; Primikirios and Roubelakis-Angelakis 2001). QTL analyses have identified regions associated with Fe and Na tolerance and Mg deficiency. For Fe tolerance, a major QTL located on chromosome 13 explained approximately 50% of the phenotypic variation in root and shoot biomass over two years. Minor effect QTL were detected on chromosomes 5, 9, 18, 19 with variation evident between years (Bert *et al.* 2013). An interspecific-hybrid population between two rootstocks was evaluated for leaf sodium exclusion. Na leaf concentrations were found to be associated with a block of 538 genes located on chromosome 11 explaining 72% of the variation (Henderson *et al.* 2017). The authors characterized four of these genes, high-affinity potassium transporters, and found allelic variants affected Na accumulation. For Mg deficiency, symptoms and Mg concentrations had a correlation of -0.52. The authors determined

deficiency was controlled by a major QTL accounting for approximately 55% of the variation located on linkage group 11 (Mandl *et al.* 2006). Based on the unstable inheritance in later generations, it was postulated that highly symptomatic plants were the result of an interaction between alleles from both progenitors. However, this study did not take into account levels of other elements such as P, K, and Ca which are known to affect Mg absorption and allocation.

In grape, few studies have examined the genetics of nutrient absorption and its relationship to sodium despite its importance in plant development and fruit quality. Mapping families remain a useful tool for understanding the genetic architecture of complex traits, such as nutrient balance, and we observed symptoms initially believed to be Mg over-accumulation in an F_1 breeding population. Leveraging the structure of this F_1 population, the objectives of this study were to determine the heritability and segregation, identify genomic regions associated with magnesium, sodium, and other macro and micro nutrients accumulation in *Vitis,* and compare SNP detection across two reference genomes.

Methods

Material and nutrient analyses

Two hundred forty-nine seedlings of a *Vitis vinifera* F_1 breeding population derived from 'Verdejo' x 'Gewürztraminer' (VxT) were transplanted in June 2013 into a research plot in Ripperdan, CA (soil type = Cajon loamy sand, Dinuba-El Peco fine sandy loam, Pachappa sandy loam, slightly – moderately saline-alkali; pH = 7.9). Row spacing was set at 1.22 m with 2.44 m between rows. Seedlings were trained and managed according to standard grower practices. Plants were fertilized with N, P, and K at rates of 14.5, 18.4, and 12.87 kg/hectare, respectively in 2015 and 18.1 (N), 23.10 (P), and 16.1 (K) kg/hectare in 2016 according to industry standard practices. Plants were visually assessed for nutrient imbalance symptoms in August (2015) and September (2016) using a 1 (present) or 0 (absence) rating where symptoms were plant stunting and/or leaf chlorosis (Figure 1). At the end of the study, a subset of symptomatic and asymptomatic vines were removed and were evaluated for root stunting. Whole leaf (petiole and blade) samples were collected from each vine, air dried, and submitted to A & L's Western Labs (Modesto, CA) for nutrient analyses in October 2015 and in October 2016. Nutrient concentrations were measured for nitrogen (N), sulfur (S), phosphorus (P), potassium (K),

magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), aluminum (Al), manganese (Mn), boron (B), copper (Cu), and zinc (Zn). N, S, P, K, Mg, Ca, and Na were reported as a percent of dry matter (% dm). Fe, Al, Mn, B, Cu and Zn were reported as parts per million (ppm).

Statistical analysis

Data were analyzed using JMP v12 statistical software (SAS Institute, Cary, NC) for normality, analysis of variance (ANOVA), and correlations. Plant symptoms were analyzed as marginal chlorosis only, stunting only, or combined (stunting and/or chlorosis). Data for Zn, Na, P, and Mn were log transformed, S, Mg, Fe, B, and Al were log10 transformed, and Ca and K were square root transformed to fulfill assumptions of normality. Significant differences in nutrient concentrations were determined using Tukey's Honest Significant Difference (HSD) ($P \geq 0.05$). Correlations were determined using Pearson's correlation coefficient (r). Hierarchical clustering was determined using the Ward method on standardized data. Broad sense heritability (H) was calculated based on mean square values across years according to Fehr (1987) with confidence intervals estimated by Knapp *et al.* (1985).

Genotyping by sequencing and QTL mapping

Young grape leaves were collected from each F_1 progeny grapevine in July 2015 and genomic DNA extracted using the Qiagen genomic DNA extraction kit (Qiagen, Inc Valencia, CA). Genomic DNA was sent to the UC Davis Genome Center's DNA Technologies and Expression Analysis Cores (University of California, Davis) for quality analysis, restriction enzyme digestion, library preparation and Illumina Hi-seq 3000 sequencing. Sequencing coverage was approximately 2.7 million reads per sample. Genotyping by sequencing (GBS) data was analyzed using the Tassel 5.0 GBSv2 pipeline (Bradbury *et al.* 2007). Quality (≤ 20) and length (≥ 20 bp) filtered and trimmed reads were aligned to the Thompson Seedless genome (Genova *et al.* 2014; Patel *et al.* 2018) and the Pinot noir 12xv2 genome (Canaguier et al., 2017) using BWA (Li and Durbin 2010). The table grape/raisin genome of Thompson Seedless was used, in addition to the wine grape-derived inbred genome of Pinot noir, to capture some of the variability in SNP detection using a reference genome. Identified SNPs were further filtered for frequency of minor (0.20) and major (≥ 0.35) allele frequencies, missing data ($\geq 10\%$), and sequencing depth (< 5 reads) using vcftools 0.1.15 (Danecek *et al.* 2011). A panel of 10,122 and 4,000 filtered SNPs

(Thompson Seedless and Pinot noir, respectively) were used for genome-wide association studies (GWAS) analyses. A kinship matrix was estimated in Tassel and used in a MLM (mixed linear model) implemented in the software GAPIT v2 for nutrient trait analyses within R statistical analysis software (Lipka *et al.* 2012; Tang *et al.* 2016, R Core Development Team, 2017). For binary traits (stunting, chlorosis, and combined symptoms), 2 principle components with P3D were used for analyses implemented within Tassel. Significance of a SNP was based on a *P* value ≤ 0.05 and a false discovery rate (FDR) ≤ 0.05. Functional annotation of genes associated with SNPs was determined using Blast2GO based on the nonredundant database from NCBI, and protein databases from Uniprot and Swissprot (Gotz et al., 2008; accessed June 2018).

Results

Field symptoms

There were 63 and 47 individual vines exhibiting symptoms, while 186 and 192 did not exhibit symptoms in 2015 and 2016, respectively, roughly following a 3:1 segregation. The parents, not grown at the time of this study, had not previously displayed any symptoms of nutrient imbalances at this location under similar fertilization regimes. Symptoms observed in the F_1 progeny included leaf, internode, and petiole stunting, as well as marginal leaf chlorosis and necrosis (Fig 1). A subset of symptomatic and asymptomatic plants evaluated for root stunting showed no visible differences (*data not shown*). Presence of symptoms (stunting or chlorosis) was consistent between 2015 and 2016 for most vines (> 90%). Only eighteen vines had symptoms in 2015, but were asymptomatic in 2016. Another two genotypes had no symptoms in 2015, but were symptomatic in 2016. When each symptom was evaluated individually, stunting and marginal chlorosis symptoms were consistent among plants in both years (> 80%).

Nutrient compilation

For the population, significant differences were detected in nutrient concentrations between 2015 and 2016 (Table 1). Large increases in Al, Fe, Mg, Zn, Mn and Ca concentrations were observed in leaf tissue between samples collected in 2015 and 2016. A decrease in N, P, K, and B leaf nutrient concentrations was observed from 2015 to 2016. When symptomatic and asymptomatic plants were analyzed separately, differences in nutrient concentration were detected in 2015 and

2016 (Table 2). Higher levels of Mg, Na, Al, and Fe were observed in symptomatic plants in both years, while a decrease in N was observed. Broad sense heritability was not estimable for Na, P, Cu, Mn, N, S, and Ca in 2015 or 2016. For Mg (H= 0.34; Confidence intervals (CI): 0.18-0.46), B (H= 0.44; CI: 0.32-0.55), K (H= 0.21; CI: 0.02-0.36), Al (H= 0.12; CI: 0 – 0.28), and Fe (H= 0.27; CI: 0.10 – 0.41) broad sense heritability was moderate to low.

Nutrient concentration and symptom correlations

Symptoms (marginal chlorosis, stunting or both) were positively correlated with Na, Mg, Fe and Al concentrations, and negatively correlated with N across both years (Table 3, Supplemental Figure 1). In 2015 and 2016 concentrations of Mg ($r \geq 0.6146$) and Al ($r \geq 0.4805$), respectively had the highest correlation with observed vineyard symptoms (Table 3). Na and Fe concentrations were also correlated with symptoms in both years, though at lower r values. Correlation with symptoms were observed for other nutrients, but were not consistent between years. In 2015, a significant negative correlation between S concentration (22%) and symptoms was observed, but not in 2016. In 2016, there was a significant negative correlation between P, Cu, and K content and symptoms (Supplemental Table 1). Other significant correlations among nutrients included strong ($r \geq 0.300$) positive correlations between N, P, and S in 2015 and 2016 and S, P, and K in 2016 and 2016 (Supplemental Table 1). Similarly, a strong positive correlation was also detected for both Mn and Ca with Mg in both years. Nutrient ratios (P/K and Mg/Ca) were examined between years for potential significant correlations with symptoms, but were not consistent between years (Supplemental Table 2).

Marker-trait associations

Using GWAS, significant associations between SNPs on chromosome 3 and Mg concentration were detected in 2015 for both the Thompson Seedless and Pinot noir genomes. SNP effects ranged from 7 to 10% (Supplemental Table 3). No SNPs associated with Mg accumulation were identified in 2016 with either genome at the $P = 0.05$ FDR level. Genic SNPs associated with Mg concentration were co-associated with SNPs identified for marginal leaf chlorosis and stunting (Supplemental Tables 4 and 5). In the Thompson genome, two additional SNPs (S3_21825966 and S3_21825918), 48 bp apart, located in a ~ 7,000 bp gene desert, explained ~ 10% of the variation associated with symptoms in 2015 and 2016. A BLAST search of the region

did not identify any significant alignments with any genes (predicted, putative or known) in *Vitis* or other species.

For Na in 2015, six SNPs were detected in 2015 on chromosomes 11, 13, and 18 in the Thompson genome. In 2016, SNPs associated with Na concentration were detected on chromosomes 11, 12, and 18 explaining 7 to 10% of the observed variation. Three of the SNPs detected (S11_16152632, S18_25745134, S18_25745143) were consistent between years. None of the SNPs associated with Na concentration were located in predicted or known genes. Only a single SNP identified in 2016 (S11_16152632) was also associated with stunting and general symptoms (Supplemental Table 3). In the Pinot noir genome, 11 SNPs were detected across chromosomes 3, 11, 15, and 17 in 2016. Individual SNPs explained 6 to 11% of the variation observed. Two of the SNPs on chromosomes 3 and 11 were also associated with Na concentrations in 2015, but were not significant when including an FDR of 0.05. Using the Pinot noir genome annotation, 8 genes were associated with Na accumulation. These included genes putatively involved metabolism and transport. Four of the gene had no functional annotation ascribed (Table 5). One genes, Vitvi11g01139, was associated with Na accumulation and annotated as a Clathrin assembly protein, which are a class of proteins involved in macromolecule transportation.

For marginal chlorosis, individual SNP effects on the Thompson Seedless genome ranged from 4 to 11%. Highest effect SNPs were detected on chromosome 3, with smaller SNPs identified on chromosomes 1,2, 4, 8, 10, 12, 16, 17, 18, 19 (2015) and 1, 10, 11, 12, 14, 17, and 18 (2016). Moderate overlap (27 out of 49) between years was evident in genic SNPs detected (Supplemental Table 4). Many of the SNPs identified in only a single year were located in genes with multiple SNPs associated with the trait. When aligned to the Pinot noir genome, marginal chlorosis was primarily associated with SNPs on chromosome 3 with minor SNPs on 6, 9, 13, 16, 17 and 19. Individual SNPs explained 4 to 12% of the variation. Similar replication of SNPs was observed when using the Thompson Seedless genome, twenty-nine SNPs were consistent across both years on chromosomes 3 and 16 and most unique SNPs were present in 2016.

For stunting, SNP effects varied from 4 up to 10% in the Thompson Seedless genome. Highest effect SNPs were detected on chromosome 3 with smaller effect SNPs located on chromosomes 1, 10, 11 and 18 (2015) and 1, 2, 4, 10, 11, 12, 15, 16, 17, and 18 (2016). Twenty-nine SNPs out of a potential 59 were shared between years. The majority of single-year SNPs were identified in 2016 (23 SNPs). When symptoms were combined, 28 SNPs were shared across both years, and 29 were only identified in a single year (Supplemental Table 3). Using the Pinot noir genome, 20 SNPs were only detected in a single year, with the majority identified in 2016 (15). Twenty-nine SNPs found on chromosome 3 were shared between years 1 and 2. Individual SNPs explained 4 to 13% of the variation observed.

When aligned to the Thompson Seedless genome, symptom-associated SNPs located within genes shared across years were predominantly found on chromosome 3 with a few additional SNPs located on chromosomes 11 and 18 (Table 4, Supplemental Table 4). Single year SNPs, including those associated in genes, were identified on chromosome 3, 4, 10, 11, 12, 15, 16, 17, and 18 (Supplemental Table 4). When aligned to the Pinot noir genome, significant SNPs were detected on chromosomes 3, 6, 11, 16, and 19. Individual SNPs explained 4 to 13% of the observed variation. Most of the identified SNPs were located on chromosome 3, and 21 were shared between 2015 and 2016 (Table 5, Supplemental Table 5).

No ion transport pathways were associated with symptom-associated SNPs based on the Thompson Seedless annotation, however approximately 50% of the genes had putative catalytic activity and 50% had binding activity (Supplemental Figure 2). None of the genes identified across both years and associated with symptoms were putative transporters, but were instead involved in processes such as oxidation, transcription, development, and stress response (Table 4). When symptom-associated SNPs located in genes based on the Pinot noir annotation were evaluated for putative activity, stress response, transcription, growth and development, and metabolic pathways were all represented similar to the Thompson genome. In addition, SNPs were also detected in several genes related to sugar and nutrient transport. One SNP associated with leaf stunting in both years and symptoms was associated with Calcium ion binding (Vivi03g00243).

Discussion

Proper macro and micro nutrient accumulation in grapevines is a perennial concern for growers, particularly in regions with marginal soils. Deficiencies, overaccumulations, or mis-partitioning of nutrients can result in economic losses in yield and fruit quality, and occasionally cause plant death. In our study, a *Vitis vinifera* F_1 population ('Verdejo' x 'Gewürztraminer'; denoted as VxT) segregating for foliar symptoms was evaluated for micro and macro nutrient and ion (N, S, P, K, Mg, Ca, Na, Fe, Al, Mn, B, Cu, and Zn) concentrations and symptom-associated SNPs.

Normal nutrient ranges for plants vary depending on environment, variety, maturity, tissue, plant age, and developmental stage making comparisons among studies difficult even when using the same cultivar (Benito *et al.* 2013; Pradubsuk and Davenport 2010; Conradie 1992; Schreiner *et al.* 2006; Schreiner 2016). In the VxT population, P, B, and Cu concentrations were within normal limits for *V. vinifera* (Bates and Wolf 2008) and had no correlation with observed physiological symptoms. All other nutrients or ions evaluated were outside of normal ranges or baseline levels have not been established. Concentrations of N, Mg, Na, Fe, and Al were outside (higher or lower) of the normal range for grape and were strongly associated with symptoms in both 2015 and 2016. Deficiencies or surplus of several of these ions can result in chlorosis, marginal leaf burn, or stunting. However, the symptoms observed were not consistent with any single nutrient imbalance or "acidic soil sickness", a term used to describe foliar symptoms related to deficiencies in Ca, Mg, or P from low pH soils (Wilcox *et al.* 2015). This suggests the symptoms in the VxT population were the result of misaccumulation in more than one ion.

Iron deficiency and aluminum toxicity can result in interveinal chlorosis and necrosis, but not the marginal leaf burn, stunting and chlorosis observed in the VxT population. In our work, strong positive correlations (<95%) were observed among Fe and Al concentrations in both symptomatic and asymptomatic plants across years. A similar positive correlation was detected in maize, but has not been reported in other crops (Hoffer and Trost 1923). Previous studies have shown that, aluminum tolerance variability exists among grape cultivars, with highly sensitive genotypes showing reduced root growth (Cancado *et al.* 2009). Conflicting information exists on the effects of aluminum on accumulation and distribution of nutrients in plants. It has been

shown that it can negatively impact plant health by restricting the uptake of nutrients predominantly Ca and Mg in maize (Mariano and Keltjens 2005). However, other studies on maize have shown that Mg and Ca content in the shoots show little variability when exposed to Al in the soil (Lidon *et al.* 2000; Olivares *et al.* 2009). In our study, high concentrations of Mg were observed despite the high concentrations of Al also being present.

In grape, Mg deficiency symptoms are typically interveinal chlorosis starting at the leaf edge. Mg overaccumulation has not been described in grape, but in other plant species was characterized by stunted growth and foliar yellowing. In our population, marginal, but not interveinal, chlorosis and stunting were observed and positively associated (32-60%) with an increase in foliar Mg content. In excess, Mg can inhibit the absorption of other essential nutrients such as Ca or K affecting root and shoot growth (Kobayashi *et al.* 2005; Tang *et al.* 2015; Venkatesan and Jayaganesh, 2010). This was similar to our study, where calcium and manganese levels decreased while Mg concentration increased in symptomatic plants. SNPs associated with Mg accumulation were identified on chromosome 3, but none of the genic SNPs were associated with putative transporters and the two highest effect SNPs were not located in a known genic region. A previous study by Mandl et al. (2006) determined that Mg deficiency was associated with a region on chromosome 11. In our work, chromosome 11 was associated with Na, but not Mg accumulation. These data combined would suggest that Mg accumulation in the VxT population is not a result of an overexpression of a Mg-specific transporter as was postulated by Mandl et al (2006). SNPs associated with foliar symptoms were also predominantly located on chromosome 3, suggesting that Mg content had a role in the visible symptoms. However, many of the remaining symptom-related SNPs did not overlap with those associated with Mg content indicating that this is only one small piece of the equation.

In grape, Na stress symptoms can include internode and leaf stunting, as well as leaf burns (Sinclair and Hoffman, 2003). Leaf chlorosis, observed in our study, is not considered a symptom of salt stress in grape, but Na levels were consistently associated with symptoms in years 1 and 2 (Baneh *et al.* 2014). This was further supported by the identification of an Na-associated SNP shared with stunting symptoms, though only in 2016. Strong correlations

between Na concentrations and those of Mg, Ca, and N were observed in the first year of this study, but were not consistent across years.

In the VxT population, Na accumulation was found to be associated with SNPs located on chromosome 11 consistent with previous work and chromosome 3 (Henderson et al., 2017). In our study, individual SNPs (genic and non-genic) did not explain a large portion of the variation observed (5 to 12%). This is similar to many GWAS studies where identified SNPs have only minor effects (Wang *et al.* 2015). Overlap between Mg and Na concentration-associated SNPs and those associated with symptoms (marginal leaf chlorosis, stunting or both) indicate that symptoms were, in part, tied to the accumulation or mispartitioning of both Mg and Na in the vine. The effect of individual SNPs varied suggesting that nutrient-related symptoms in this population may be the result of interactions of various ions, particularly Al, Na, Fe, and Mg.

When comparing SNP results between the two genomes used in this study, it was clear that chromosome 3 was a major contributor of the phenotypic variation observed in the VxT population. Similarly, individual SNPs identified in both genomes had small effects on symptoms (leaf stunting and/or chlorosis), with few genes having more than one significant SNP. In the Thompson genome annotation, one Na associated SNP was shared with symptom associated SNPs, and most Na associated SNPs were located in genic “deserts”. However, in the Pinot noir genome v3 annotation, one Na associated SNP was also associated with symptoms in both years, and located within a gene (Vitvi11g01681). These data highlight the importance of genome and annotation selection when performing these types of studies.

In summary, we evaluated a *Vitis vinifera* segregating population for micro and macro nutrient accumulation across two years. Broad sense heritability was low for most nutrient concentrations, indicating a large environmental component. This was further evident in that specific nutrient concentrations fluctuated with vine age from 2015 to 2016, though trends were consistent across years. Symptom-associated genic SNPs identified were located in putative stress response-related genes. However, many SNPs identified were not associated within known genic regions. Many of the SNPs associated with Mg accumulation were distributed across chromosome 3 for both of the genomes evaluated. While it is clear that a block of SNPs on

chromosome 3 is affecting this trait, current resources were insufficient to identify associated genes. As more grape genomes are sequenced, it is quite apparent that genomic inversions and deletions are common among cultivars and the grapevine gene annotation is constantly being modified. Some of the candidate SNPs identified here may associate with currently unannotated genes not present in the Thompson Seedless or Pinot noir genomes. Additionally, as 'Geuwurztraminer' is an aromatic sport of 'Traminer', which itself is the grandparent of 'Verdejo', this population is genetically similar to an F_2 back cross 1 (F_2BC_1). Grape is particularly susceptible to inbreeding depression, and these SNPs may be associated with deleterious alleles of regulatory or genic regions not annotated in sequenced grape genomes. SNPs associated with Na and Mg accumulation as well as foliar symptoms were identified. However, imbalances in neither of these single ions were able to fully explain the observed symptoms, and the relationship with symptoms varied as the plants aged and other nutrient levels changed. These fluid relationships highlight the complexity of micro- and macro nutrient relationships in perennial crops.

Acknowledgements

The authors would like to thank Elisha Partin and Lindsay Wourms for technical assistance in sample collection and in-field phenotyping. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer. Funding for this project was provided, in part, by E. & J. Gallo Winery. The sequencing was carried out at the DNA Technologies and Expression Analysis Cores at the UC Davis Genome Center, supported by NIH Shared Instrumentation Grant 1S10OD010786-01.

References

Arif N, Yadav V, Singh S, Singh S, Ahmad P, Mishra RK, Sharma S, Tripathi DK, Dubey NK, Chauhan DK. 2016. Influence of high and low levels of plant-beneficial heavy metal ions on plant growth and development. Front Environ Sci 21:69.

Baneh HD, Hassani A, Shaieste FG. 2014. Effects of salinity on leaf mineral composition and salt injury symptoms of some Iranian wild grapevine (*Vitis vinifera* L. ssp. *sylvestris*) genotypes. J Int Sci Wine and Vine 48:1692.

Bates T, Wolf T. 2008. Vineyard Nutrient Management. Wolf T. (eds) In Wine Grape Production for Eastern North America. NRAES, Ithaca NY

Benito A, Romero I, Dominguez N, Garcia-Escudero E, Martin I. 2013. Leaf blad and petiole analysis for nutrient diagnosis in *Vitis vinifera* L. cv. Garnacha tinta. Aus j Grape Wine Res 19:285-298.

Bernstein L. 1975. Effects of salinity and sodicity on plant growth. Ann. Rev. Phytopath. 13:295-312.

Bert PF, Bordenave L, Donnart M, Hevin C, Ollat N, Decroocq S. 2016. Mapping genetic loci for tolerance to lime-induced iron deficiency chlorosis in grapevine rootstocks (*Vitis* sp.) Theo Appl Genet 126:451-473.

Bose J, Babourina O, Rengel Z. 2011. Role of magnesium in alleviation of aluminum toxicity in plants. J Exp Bot 63:2251-2264.

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635.

Brancadoro L, Rabotti G, Scienza A, Zocchi G. 1995. Mechansims of Fe-efficiency in roots of *Vitis* spp. in response to iron deficiency stress. Plant and Soil 2:229-234.

Canaguier A, Grimplet J, Di Gaspero G, Scalabrin S, Duchene E, Choisne N, Mohellibi N, Guichard C, Rombauts S, Le Clainche I, Berard A, Chauveau A, Bounon R, Rustenholz C, MOrgante M, Le Paslier MC, Brunel D, Adam-Blondon AF. 2017. A new version of the grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). Genom Data 14:56-62.

Cancado GMA, Ribeiro AP, Pineros MA, Miyata LY, Alvarenga AA, Villa F, Pasqual M, Purgatto E. 2009. Evaluation of aluminium tolerance in grapevine rootstocks. Vitis 48:167-173.

Carbonell-Barrachina AA, Burlo-Carbonell F, Mataix-Beneyto J. 2008. Effect of sodium arsenite and sodium chloride on bean plant nutrition (macronutrients). 20:1617-1633.

Christensen P. 1984. Nutrient level comparisons of leaf petioles and blades in twenty-six grape cultivars over three years (1979 through 1981). Am J Enol Vit 35:124-133.

Clemens S. 2001. Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212:475-486.

Conradie WJ. 1981. Seasonal uptake of nutrients by Chenin Blanc in sand culture: II. phosphorous, potassium, calcium and magnesium. S. Afr. J. Enol. Vit 2:7-13.

456 Conradie WJ. 1992. Partitioning of nitrogen by the grapevine during autumn and the utilization of
457 nitrogen reserves during the following growing season. S African J Enol Vit 13:45-51.
458
459 Conradie WJ, Saayman D. 1989. Effects of long-term nitrogen, phosphorus, and potassium
460 fertilization on Chenin Blanc vines. II. Leaf analyses and grape composition. Amer J Enol Vit
461 40:91-98.
462
463 Cordovilla MP, Ocana A, Libero F, Lluch C. 1995. Salinity effects on growth analysis and
464 nutrient composition in four grain legumes – rhizobium symbiosis. J Plant Nut 18:1595 –
465 1609.
466
467 Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker R, Lunter G,
468 Marth G, Sherry ST, McVean G, Durbin R, 1000 Genomes Project Analysis Group. 2011. The
469 variant call format and VCFtools. Bioinformatics. 27:2156-2158.
470
471 Davies C, Shin R, Liu W, Thomas MR, Schachtman DP. 2006. Transporters expressed during grape
472 berry (*Vitis vinifera* L.) development are associated with an increase in berry size and berry
473 potassium accumulation. J Exp Bot 57:3209-3216.
474
475 Downton WJS. 1977. Chloride accumulation in different species of grape-vine. Sci Hort 7:249-253.
476
477 Fehr WR. 1987. Principles of cultivar development. Macmillan, Inc. New York.
478
479 Genova AD, Almeida AM, Munoz-Espinoza C, Vizoso P, Travisany D, Moraga C, Pinto M, Hinrichsen
480 P, Orellana A, Maass A. 2014. Whole genome comparison between table and wine grapes reveals a
481 comprehensive catalog of structural variants. BMC Plant Biology 14:7.
482
483 Gong HJ, Blackmore DH, Clingeleffer PR, Sykes SR, Walker RR. 2015. Variation for
484 potassium and sodium accumulation in a family from a cross between grapevine rootstocks
485 K 51-40 and 140 Ruggeri. J Grape Res 53:65-72.
486
487 Gotz S, Garcia-Gomez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talon M,
488 Dopazo J, Conesa A. 2008. High-throughput functional annotation and data mining with the
489 Blast2GO suite. Nucl Acid Res 36:3420-3435.
490
491 Grattan SR, Grieve CM. 1992. Mineral element acquisition and growth response of plants
492 grown in saline environments. Ag Eco Env 38:275-300.
493
494 Guo W, Nazim H, Liang Z, Yang D. 2016. Magnesium deficiency in plants: An urgent
495 problem. Crop Journal 4:83-91.
496
497 Henderson SW, Dunlevy JD, Wu Y, Blackmore DH, Walker RR, Edwards EJ, Gillihma M,
498 Walker AR. 2017. Functional differences in transport properties of natural HKT1;1 variants
499 influence shoot Na+ exclusion in grapevine rootstocks. New Phyto 217:1113-1127.
500

Hermans C, Verbruggen N. 2005. Physiological characterization of Mg deficiency in *Arabidopsis thaliana*. J Exp Bot 56:2153-2161.

Hoffer GN, Trost JF. 1923. The accumulation of iron and aluminum compounds in corn plants and its probable relationship to root rots. II. J Amer Soc Agron 15:323-331.

Hu X, Boyer GL. 1996. Siderophore-mediated aluminum uptake by *Bacillus megaterium* ATC 19213. Appl Env Micr 62:4044-4048.

Jimenez S, Gogorcena Y, Hevin C, Rombola AD, Ollat N. 2007. Nitrogen nutrition influences some biochemical responses to iron deficiency in tolerant and sensitive genotypes of *Vitis*. Plant and Soil 290:343-355.

Knapp SJ, Stroup WW, Ross WM. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 25:192-194.

Kobayashi H, Masaoka Y, Sato S. 2005. Effects of excess magnesium on the growth and mineral content of rice and *Echinochloa*. Plant Prod Sci 8:38-43.

Kocsis L, Walker MA. 2003. Screening *Vitis* species for use in breeding magnesium tolerant rootstocks. Acta Hort 603:10.17660/ActaHortic.2003.603.52

Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler Transform. Bioinformatics, Epub. [PMID: 20080505]

Lidon FC, Azinheira HG, Barreiro MG. 2000. Aluminum toxicity in maize: biomass production and nutrient uptake and translocation. J Plant Nutr. 23:151-160.

Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES, Zhang Z. 2012. GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397-2399.

Ma JF, Chen ZC, Shen RF. 2014. Molecular mechanisms of Al tolerance in gramineous plants. Plant and Soil 381:1-12.

Maathius FJM. 2009. Physiological functions of mineral macronutrients. Curr Op Plant Bio 12:250-258.

Maathuis FJM. 2013. Sodium in plants: perception, signaling, and regulation of sodium fluxes. J Exp Bot 65:849-858.

Mandl K, Santiago JL, Hack R, Fardossi A, Regner F. 2006. A genetic map of Welschriesling x Sirius for the identification of magnesium-deficiency by QTL analysis. Euphytica 149:133-144.

Mariano ED, Keltjens WG. 2005. Long-term effects of aluminum exposure on nutrient uptake by maize genotypes differing in aluminum resistance. J Plant Nut 28:323-333.

Mohammadkhani N, Abbaspour N. 2015. Salinity effects on potassium accumulation and transporters expression in grape (*Vitis vinifera*). Iran J Plant Phys 5:1483-1494.

Morris JR, Sims CA, Cawthon DL. 1983. Effects of excessive potassium levels on pH, acidity, and color of fresh and stored grape juice. Am J Enol Vit 34:35-39.

Mossor-Pietraszewska T. 2001. Effect of aluminum on plant growth and metabolism. Acta Biochimica Polonica 48:673-686.

Mpelasoka BS, Schachtman DP, Treeby MT, Thomas MR. 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aus J Grape Wine Res 9:154-168.

Naranjo-Arcos MA, Maurer F, Meiser J, Pateyron S, Fink-Straube C, Bauer P. 2017. Dissection of iron signaling and iron accumulation by overexpression of subgroup Ib bHLH039 protein. Sci Reports 7:10911.

Olivares E, Pena E, Marcano E, Mostacero J, Aguiar G, Benitez M, Rengifo E. 2009. Aluminum accumulation and its relationship with mineral plant nutrients in 12 pteridophytes from Venezuela. Environ Exp Bot 65:132-141.

Patel S, Lu Z, Jin X, Swaminathan P, Zeng E, Fennell AY. 2018. Comparison of three assembly strategies for a heterozygous seedless grape genome assembly. BMC Genomics 19:57.

Perez-Castro R, Kasai K, Gainza-Cortes F, Ruiz-Lara S, Casaretto JA, Pena-Cortes H, Tapia J, Fujiwara T, Gonzalez E. 2012. *VvBOR1*, the grapevine ortholog of *AtBOR1*, encodes an efflux boron transporter that is differentially expressed throughout reproductive development of *Vitis vinifera* L. Plant and Cell Phys. 53:485-494.

Poor D, Pedo S, Bertoldi D, Bortolotti L, Failla O, Zamboni M. 2013. Evaluation of new rootstocks for grapevine: nutritional aspects. Proc VIIth IS Min. Nut. Fruit Crop Eds.: Poovarodom and Yingjajaval S. Acta Hort. 984, ISHS.

Pradubsuk S, Davenport JR. 2010. Seasonal uptake and partitioning of macronutrients in mature 'Concord' grape. J Amer Soc Hort 135:474-483.

Primikiros NI, Roubelakis-Angelakis KA. 2001. Indications for post-translational regulation of *Vitis vinifera* L., arginine decarboxylase. Plant Molec Biol 45:669-678.

R Core Development Team. 2017. R Foundation for Statistical Computing Vienna, Austria. https://www.R-project.org/

Rashad RT, Hussein RA. 2014. A comparison study on the effect of some growth regulators on the nutrients content of maize plant under salinity conditions. Ann Ag Sci 59:89-94.

592 Rogier SY, Keller M, Holzapfel BP, Virgona JM. 2000. Accumulation of potassium and
593 calcium by ripening berries on field vines of *Vitis vinifera* (L) cv. Shiraz. Aus J Grape Wine
594 Res 6:240-243.
595
596 Schreiner RP, Scagel CF, Baham J. 2006. Nutrient uptake and distribution in a mature 'Pinot
597 noir' vineyard. HortSci 41:336-345.
598
599 Schreiner RP. 2016. Nutrient uptake and distribution in young Pinot noir grapevines over
600 two seasons. Amer Soc. Enol Vit 67:436-448.
601
602 Shani U, Ben-Gal A. 2005. Long-term response of grapevines to salinity: Osmotic effects and
603 ion toxicity. Am J Enol Vitic 56:148-154.
604
605 Shaul O. 2002. Magnesium transport and function in plants: the tip of the iceberg. BioMetals
606 15:309-323.
607
608 Sinclair C, Hoffman AA. 2003. Monitoring salt stress in grapevines: are measures of plant
609 trait variability useful? J Appl Ecol 40:928-937.
610
611 Skinner PW, Matthews MA. 1990. A novel interaction of magnesium translocation with the
612 supply of phosphorous to roots of grapevine (*Vitis vinifera* L.) Plant Cell Env 13:821-826.
613
614 Spiers JM, Braswell JH. 1994. 793 PB 039 Calcium, magnesium, and nitrogen fertilization
615 affects leaf nutrient content and growth of 'Sterling' muscadine grape. HortSci 29:546.
616
617 Tagliavini M, Romboloa D. 2001. Iron deficiency and chlorosis in orchard and vineyard
618 ecosystems. E J Ag 15:71-92.
619
620 Tang RJ, Zhao FG, Garcia VJ, Kleist TJ, Yang L, Zhang HX, Luan S. 2010. Tonoplast CBL-CIPK
621 calcium signaling network regulates magnesium homeostasis in *Arabidopsis*. PNAS
622 112:3134-3139.
623
624 Tavakkoli E, Rengasamy P, McDonald GK. 2010. High concentrations of Na+ and Cl- ions in
625 soil solution have simultaneous detrimental effects on growth of faba bean under salinity
626 stress. J Exp Bot 61:4449-4459.
627
628 Thenabadu MW. 1968. Magnesium-sodium interactions effecting the uptake and
629 distribution of potassium and calcium by cotton. Plant and Soil 29:132-143.
630
631 Venkatesan S, Jayaganesh S. 2010. Characterization of magnesium toxicity, its influence on
632 amino acid synthesis pathway and biochemical parameters of tea. Res J Phytochem 4:67-
633 77.
634
635 Verbruggen N, Hermans C. 2013. Physiological and molecular responses to magnesium
636 nutritional imbalance in plants. Plant and Soil 368:87-99.
637

638 Volkmar KM, Hu Y, and Steppuhn H. 1998. Physiological responses of plants to salinity: A
639 review. Can J Plant Sci 78:19-27.
640
641 Wang L, Shen H, Liu H, Guo G. 2015. Mixture SNPs effect on phenotype in genome-wide
642 association studies. BMC Genomics 16:3
643
644 Wilcox WF, Gubler WD, and Uyemoto JK. 2015. Compendium of grape diseases, disorders,
645 and pests. 2nd Ed. Pp.159-167. APS Press St. Paul, MN.
646
647 Williams CMJ, Maier NA, Bartlett L. 2004. Effect of molybdenum foliar sprays on yield, berry
648 size, seed formation, and petiolar nutrient composition of "Merlot" grapevines. J Plant Nut
649 27:1891-1916
650
651 Zhang Z. 2016. GAPIT version 2: an enhanced integrated tool for genomic association and
652 prediction. Plant Genome 9:2
653
654 Zhu JK, Liu J, Xiong L. 1998. Genetic analysis of salt tolerance in Arabidopsis: Evidence for a
655 critical role of potassium nutrition. Plant Cell. 10:1181-1191.
656

Figure 1

Figure 1. Symptomatic (stunted) and asymptomatic grape stem internodes (A) and leaves (B) from an F_1 *Vitis* population. From left to right (C), asymptomatic and symptomatic (marginal leaf chlorosis and burn) leaves.

Figure 1. Symptomatic (stunted) and asymptomatic grape stem internodes (A) and leaves (B) from an F_1 *Vitis* population. From left to right (C), asymptomatic and symptomatic (marginal leaf chlorosis and burn) leaves.

Figure 2

Figure 2. Manhattan plot of Single Nucleotide Polymorphisms (SNPs) associated with foliar (marginal leaf chlorosis/burn and stunting) symptoms in a *Vitis* F_1 segregating population in A. 2015 and B. 2016.

Figure 2. Manhattan plot of Single Nucleotide Polymorphisms (SNPs) associated with foliar (marginal leaf chlorosis/burn and stunting) symptoms in a *Vitis* F_1 segregating population in A. 2015 and B. 2016.

Figure 2. Manhattan plot of SNPs associated with symptoms in B. 2015 and B. 2016.

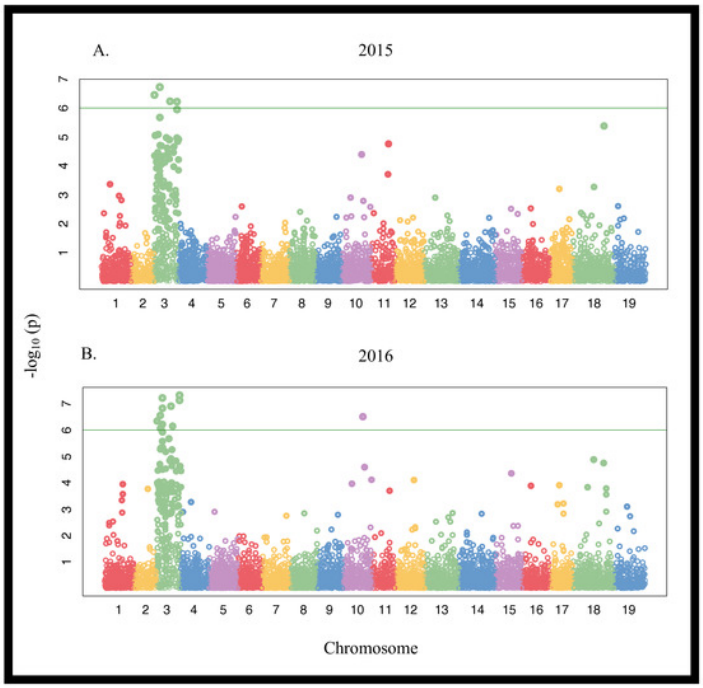

Table 1(on next page)

Table 1. Mean nutrient concentrations from combined leaf and petiole samples collected in 2015 and 2016 from an F_1 *Vitis* population.

Table 1. Mean nutrient concentrations from combined leaf and petiole samples collected in 2015 and 2016 from an F_1 *Vitis* population.

Table 1. Mean nutrient concentrations from combined leaf and petiole samples collected in 2015 and 2016 from an F_1 *Vitis* population.

Nutrient	Unit[A]	Population Mean ± (StD)		Normal range[B]
		2015	2016	(petioles)
N	% dm	2.19 ± 0.25*	1.62 ± 0.26	0.8 – 1.2
S	% dm	0.18 ± 0.02*	0.17 ± 0.03	-
P	% dm	0.17 ± 0.03*	0.13 ± 0.03	0.14 – 0.30
K	% dm	0.94 ± 0.24*	0.70 ± 0.24	1.2 – 2.0
Mg	% dm	0.69 ± 0.17*	0.83 ± 0.15	0.35 – 0.75
Ca	% dm	2.44 ± 0.44*	3.27 ± 0.56	1 – 2
Na	% dm	0.02 ± 0.02*	0.05 ± 0.03	-
Fe	ppm	400.41 ± 86.27*	484.20 ± 175.14	30 – 100
Al	ppm	238.05 ± 57.14*	315.67 ± 112.02	-
Mn	ppm	59.31 ± 13.08*	69.53 ± 18.69	100 – 1000
B	ppm	50.81 ± 14.60*	42.00 ± 16.02	25 - 50
Cu	ppm	6.20 ± 2.43	6.12 ± 1.25	5 -15
Zn	ppm	20.78 ± 3.95*	26.12 ± 5.88	30 - 60

[A] Units of measurement for each micro or macronutrient analyzed as percent dry matter (% dm) or parts per million (ppm).

[B] Typical range for petiole concentrations for *Vitis* cultivars selected from Bates and Wolf (2008).

* indicates a significant difference ($P \leq 0.05$) in concentration between 2015 and 2016.

Table 2(on next page)

Table 2. Population means for grapevine nutrient concentrations

Table 2. Population means for grapevine nutrient concentrations

Table 2. Population means for grapevine nutrient concentrations

Ion	Unit[A]	2015		2016	
		No Sym[B]	Symp	No Symp	Symp
N	% dm	2.22 ± 0.23*	2.09 ± 0.27	1.63 ± 0.26*	1.53 ± 0.22
S	% dm	0.18 ± 0.02*	0.17 ± 0.02	0.17 ± 0.03	0.17 ± 0.02
P	% dm	0.17 ± 0.03	0.17 ± 0.02	0.13 ± 0.03*	0.15 ± 0.03
K	% dm	0.93 ± 0.24	0.96 ± 0.25	0.66 ± 0.23*	0.86 ± 0.26
Mg	% dm	0.63 ± 0.11*	0.89 ± 0.19	0.80 ± 0.14*	0.95 ± 0.14
Ca	% dm	2.44 ± 0.43	2.45 ± 0.44	3.37 ± 0.54*	2.86 ± 0.45
Na	% dm	0.02 ± 0.02*	0.03 ± 0.02	0.04 ± 0.02*	0.06 ± 0.05
Fe	ppm	389.85 ± 84.44*	431.57 ± 85.68	443.28 ± 139.08*	660.06 ± 205.68
Al	ppm	230.19 ± 55.58*	261.25 ± 55.76	289.02 ± 89.17*	430.21 ± 128.03
Mn	ppm	58.61 ± 12.69	61.35 ± 14.15	70.70 ± 19.17*	64.49 ± 15.63
B	ppm	50.40 ± 14.39	52.02 ± 15.24	41.46 ± 16.61	44.34 ± 13.07
Cu	ppm	6.17 ± 2.60	6.32 ± 1.87	5.95 ± 1.20*	6.85 ± 1.20
Zn	ppm	20.96 ± 3.90	20.25 ± 4.08	25.77 ± 5.20	27.62 ± 8.11

[A] Units of measurement for each micro or macronutrient analyzed as percent dry matter (% dm) or parts per million (ppm).

[B] Non symptomatic (No sym) and Symptomatic (Symp) plants.

* indicates a significant difference between symptomatic and asymptomatic plants

Table 3(on next page)

Table 3. Multivariate analysis of correlation (r) among nutrient concentrations in 2015 (gray) and 2016 (white) from grape vines.

Table 3. Multivariate analysis of correlation (r) among nutrient concentrations in 2015 (gray) and 2016 (white) from grape vines.

Table 3. Multivariate analysis of correlation (r) among nutrient concentrations in 2015 (gray) and 2016 (white) from grape vines.

	Mg	**Ca**	**Na**	**Fe**	**Al**	**N**	**Sym[A]**
Mg	-	0.4414***	0.3175***	0.1663**	0.2220**	-0.3552***	0.6146***
Ca	0.4123***	-	0.1414*	NS	NS	-0.3394***	NS
Na	0.2134**	NS	-	0.1847*	0.1601*	NS	0.2973***
Fe	NS	-0.2168**	0.1850*	-	0.9675***	-0.1512*	0.2149**
Al	0.1385*	-0.1958*	0.1919*	0.9841***	-	-0.2290**	0.2406**
N	-0.2852*	-0.2822***	NS	NS	NS	-	-0.2275**
Sym	0.3200***	-0.3619***	0.2436***	0.4724***	0.4805***	-0.1679*	-

[A] Symptoms

*P ≤ 0.05

**P < 0.001

***P < 0.0001

NS = not significant

Table 4(on next page)

Table 4. Genic Single Nucleotide Polymorphisms (SNPs) associated with symptoms using the Thompson Sdls genome annotation in 2015 and 2016 in an F_1 population of *V. vinifera*.

Table 4. Genic Single Nucleotide Polymorphisms (SNPs) associated with symptoms using the Thompson Sdls genome annotation in 2015 and 2016 in an F_1 population of *V. vinifera*.

Table 4. Genic Single Nucleotide Polymorphisms (SNPs) associated with symptoms using the Thompson Sdls genome annotation in 2015 and 2016 in an F_1 population of *V. vinifera*.

Chr[A]	Gene[B]	SNP	Effect[C]	Putative function[D]
10	g1087	S10_19692199	6-9%	Polyphenol oxidase
3	g1405	S3_149721	9%	Probable beta-D-xylosidase 5
	g1407	S3_265316	5-6%	Uncharacterized protein LOC109124260
	g1462	S3_921272	6-7%	FAD-linked sulfhydryl oxidase ERV1
	g1519	S3_2023880	5%	HTH-type transcript regulator protein ptxE
	g1523	S3_2032939	4-5%	CASP-like protein 5C1
		S3_2032882	4-6%	
	g1599	S3_2800042	6%	Glycoside hydrolase, family 10
	g1629	S3_3258173	6-9%	At4g33990
	g1632	S3_3366650	7-9%	Oxysterol-binding protein 5
		S3_3366649	5-7%	
	g1658	S3_3935538	7-9%	Polyphenol oxidase
	g1676	S3_4305461	5%	Myb-binding protein 1A
	g1689	S3_4773185	5%	Scopoletin glucosyltransferase-like
	g1736	S3_5569343	7%	Receptor-like protein kinase HAIKU2
	g1784	S3_6826327	5-7%	Os01g0234100-like isoform X1
	g1882	S3_8415245	4-6%	Classical arabinogalactan protein 9
	g1999	S3_10174220	4-6%	Dof zinc finger protein DOF3.4-like
	g2046	S3_11599143	7-8%	CSC1-like protein HYP1 isoform X1
	g2086	S3_12359712	7%	Protein unc-50 homolog
	g2129	S3_13316810	5-6%	Serine/threonine-protein kinase BLUS1 like
	g2137	S3_13577682	6-7%	Exocyst complex component SEC6
	g2185	S3_14537967	7%	Hypothetical protein VITISV_042288
	g2192	S3_14638969	5-6%	E3 ubiquitin-protein ligase MBR2 iso X1
	g2217	S3_15151065	9-10%	Mitochondrial Rho GTPase 1-like
	g2363	S3_18838966	5-7%	DNA-directed RNA poly II, IV, V sub 3
	g2581	S3_22457867	6-7%	D-3-phosphoglycerate dehydrogenase 1 like
	g2622	S3_23241926	6%	KH domain-containing protein HEN4

[A] Chromosome

[B] Putative grape gene based on the Thompson Seedless genome (Patel et al., 2018).

[C] Percent of the variation explained by a SNP.

[D] Putative function based on BLAST2GO annotation (Gotz et al., 2008).

Table 5(on next page)

Table 5. Genic Single Nucleotide Polymorphisms (SNPs) associated with symptoms using the Pinot noir genome annotation in 2015 and 2016 in an F_1 population of *V. vinifera*.

Table 5. Genic Single Nucleotide Polymorphisms (SNPs) associated with symptoms using the Pinot noir genome annotation in 2015 and 2016 in an F_1 population of *V. vinifera*.

Table 5. Genic Single Nucleotide Polymorphisms (SNPs) associated with symptoms using the Pinot noir genome annotation in 2015 and 2016 in an F_1 population of *V. vinifera*.

Chr[A]	Gene[B]	SNP	Effect[C]	Putative function[D]
3	Vitvi03g00243	S3_2846002	6%	Calcium ion binding protein
	Vitvi03g00247	S3_2876678	4%	Tonoplast monosaccharide transporter2
	Vitvi03g00380	S3_4196400	4 - 6%	Unknown
	Vitvi03g00384	S3_4208958	5 - 7%	Integral membrane protein
	Vitvi03g00384	S3_4209015	5 - 7%	Integral membrane protein
	Vitvi03g00430	S3_4637832	5 - 7%	Dof zinc finger protein DOF5.8
	Vitvi03g00520	S3_5653914	5 - 7%	Basic helix-loop-helix (bHLH) family
	Vitvi03g00534	S3_5852953	5%	ABA-specific glucosyltransferase
	Vitvi03g00543	S3_5986778	7 - 8%	DNA-directed RNA polymerase II
	Vitvi03g00560	S3_6167883	7 - 8%	UNC-50
	Vitvi03g00583	S3_6554413	6 - 7%	TIP41
	Vitvi03g00603	S3_6823070	8 - 9%	R protein MLA10
	Vitvi03g00724	S3_8516174	3 - 4%	FKBP12-rapamycin complex-associated protein
	Vitvi03g00756	S3_9107785	5 - 6%	Pathogenesis-related protein 1B
	Vitvi03g00772	S3_9286732	9 - 14%	Transposon protein
	Vitvi03g00777	S3_9374358	6 - 7%	EMB2758 (embryo defective 2758)
	Vitvi03g00783	S3_9441309	6 - 8%	Proline iminopeptidase
	Vitvi03g00855	S3_11308538	6 - 12%	Zinc finger (C3HC4-type ring finger)
	Vitvi03g00858	S3_11368101	5%	SEC6
	Vitvi03g00925	S3_12779089	5 - 9%	Unknown
	Vitvi03g00997	S3_14475933	4 - 5%	No hit
	Vitvi03g01012	S3_14786293	5 - 6%	No hit
	Vitvi03g01067	S3_16246430	5%	MADS-box agamous-like 24
	Vitvi03g01088	S3_16701286	6 - 7%	Oxoglutarate/malate translocator DIT2.1
	Vitvi03g01092	S3_16774264	7 - 9%	GTP cyclohydrolase II
	Vitvi03g01092	S3_16774395	5 - 9%	GTP cyclohydrolase II
	Vitvi03g01518	S3_4201002	5 - 7%	PREDICTED: uncharacterized protein
	Vitvi03g01581	S3_6106914	7 - 9%	PREDICTED: PRKR-interacting protein 1
	Vitvi03g01606	S3_7076477	7 - 12%	hypothetical protein VITISV_014038
	Vitvi03g01649	S3_8895549	6 - 8%	PREDICTED: basic form of pathogenesis-related protein 1
3	Vitvi03g01769	S3_15171032	5 - 6%	PREDICTED: uncharacterized protein
	Vitvi03g01769	S3_15171570	6 - 7%	PREDICTED: uncharacterized protein
	Vitvi03g01792	S3_16473090	4 - 6%	Peru 1
11	Vitvi11g00662	S11_7516783	4%	No hit

[A] Chromosome

[B] Putative grape gene based on the Pinot Noir 12xv2 genome (Canaguier et al., 2017).

[C] Percent of the variation explained by a SNP.

[D] Functional annotation based on Pinot Noir genome v3 annotation (Canaguier et al., 2017).