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], **Cheng Zou** [3], **Peter Cousins** [4]

[1] San Joaquin Valley Agricultural Sciences Center, USDA ARS, Parlier, CA, United States

[2] Grape Genetics Unit, USDA ARS, Geneva, NY, United States

[3] Cornelll University, Ithaca, New York, United States

[4] 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. Our study evaluated micro and macro nutrient concentrations in *Vitis vinifera* L. to identify associated SNPs using an association approach with genotype by sequencing data. 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, 7 and 17 depending on the trait and genome used for analyses explaining up to 40% of the observed variation. Symptoms and magnesium concentration were primarily associated with SNPs on chromosome 3, while SNPs associated with increased sodium content were primarily found on chromosomes 11 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], Cheng Zou[3]and Peter Cousins[4]

[1] USDA ARS San Joaquin Valley Agricultural Sciences Center Parlier, CA 93648

[2] USDA ARS Grape Genetics Unit Geneva, NY

[3] Cornell University, Ithaca, NY

[4]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 vinifera* L. to identify associated SNPs using an association approach with genotype by sequencing data. 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, 7 and 17 depending on the trait and genome used for analyses explaining up to 40% of the observed variation. Symptoms and magnesium concentration were primarily associated with SNPs on chromosome 3, while SNPs associated with increased sodium content were primarily found on chromosomes 11 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.

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).

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 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). In cultivated grape, *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) , though the severity of response can vary greatly depending on the genotype used and level of excess (Kocsis and Walker 2003; Porro *et al.* 2013). However, foliar symptoms may also be the result of interactions among nutrients, and this has not been well-studied (Shikhamany *et al.* 1988; Skinner and Matthews 1990). Skinner and Mathews (1990) found that adding phosphorous to the soil eliminated Mg deficiency symptoms and increased overall Mg concentrations.

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 up to 50% of the phenotypic variation in root and shoot biomass over two years using a *Vitis* inter-specific cross between Cabernet Sauvignon (*V. vinifera*) and Gloire de Montpellier (*V. riparia*) under chlorosing conditions. Minor effect QTL were also detected on chromosomes 5, 9, 18, 19 with variation evident between years (Bert *et al.* 2013). Two QTL on chromosomes 11 and 13 were associated with Fe concentration in

grafted plants only. 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.* 2018). The authors characterized the proteins from four different alleles of high-affinity potassium transporters, and found allelic variants affected Na accumulation. For Mg deficiency, leaf symptoms and Mg concentrations were negatively correlated(r= -0.52), and it was determined that 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 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 evaluate the levels of other elements such as P, K, and Ca which are known to affect Mg absorption and allocation. Each of these studies identified QTL using inter-specific crosses.

In grape, few studies have examined the genetics of nutrient absorption and concentrations and its relationship to phenotypic variation despite 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 derived from a cross between two *V. vinifera* cultivars, 'Verdejo' and 'Gewurztraminer'. Leveraging the structure of this F_1 population, the objectives of this study were to determine the relationship between nutrients and visible symptoms, heritability and segregation, identify genomic regions associated with magnesium, sodium, and other macro and micro nutrients accumulation in *Vitis vinifera* L.*,* and compare SNP detection across two reference genomes.

Materials and 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). All vines were own rooted with no grafting. 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 12.9 kg/hectare, respectively in 2015 and 18.1, 23.1, and 16.1 kg/hectare in 2016. Fertilization was performed according to industry standard practices; fertigation by applying a liquid fertilizer solution through the drip irrigation every two weeks from the time of fruit set. Lateral shoots were removed from the trunk during establishment and vines were trained to a unilateral cordon and spur-pruned. Plants were visually assessed for foliar 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). Plant stunting and leaf chlorosis were evaluated separately. For nutrient analysis fully expanded whole leaf (petiole and blade) samples were collected from each vine. Due to variability between genotypes, equivalent leaf volume was collected, typically between 15-25 mature leaves. The leaves were sampled from fertile (fruiting) shoots into brown paper bags and air dried indoors at 22 °C. Once dry, the leaves were 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 was measured using automated combustion at 900 °C. S, P, K, Mn, Ca, Na, Fe, Al, Mg, B, Cu and Zn were measured using nitric/hydrochloric acid digestion using a microwave, analysis was by inductively coupled plasma spectrometry (ICP) as detailed by The North American Proficiency Testing Program (Black, 1965; naptprogram.org). 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). At the end of the study, a subset of symptomatic and asymptomatic vines was removed and evaluated for root stunting.

Statistical analysis

Nutrient data were analyzed using JMP v12 statistical software (SAS Institute, Cary, NC) for normality (Shapiro-Wilk W Test), analysis of variance (ANOVA), hierarchical clustering, and correlations for relationships within and between years. 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

147 nutrient concentrations between years or genotypes were determined using Tukey's Honest
148 Significant Difference (HSD) ($P \geq 0.05$). Correlations were determined using Pearson's
149 correlation coefficient (r) on the transformed data. Hierarchical clustering was determined using
150 the Ward method on standardized data. Broad sense heritability (H) was calculated based on
151 mean square values using the one location across two years formula modified from Fehr (1987)
152 by Wang et al. (2000) with confidence intervals estimated by Knapp *et al.* (1985). Best Linear
153 Unbiased Predictors (BLUPs) were calculated with the lme4 package in R (v4.0.2 R Core
154 Development Team, 2017) using nutrient concentration, genotype, and year as random effects
155 (Henderson, 1975; Liu et al, 2008; Merk, 2011). Principal component analysis for the population
156 was calculated using the BLUPs for each nutrient concentration within JMP12.0.1.
157
158 *Genotyping by sequencing and mapping of significant SNP associations*
159 Young grape leaves were collected from each F_1 progeny grapevine in July 2015 and genomic
160 DNA extracted using the Qiagen genomic DNA extraction kit (Qiagen, Inc Valencia, CA).
161 Genomic DNA was sent to the UC Davis Genome Center's DNA Technologies and Expression
162 Analysis Cores (University of California, Davis) for quality analysis, restriction enzyme
163 digestion (ApeK1), library preparation and Illumina Hi-seq 3000 sequencing. Sequencing
164 coverage was approximately 2.7 million reads per sample. Genotyping by sequencing (GBS)
165 data was analyzed using the Tassel 5.0 GBSv2 pipeline (Bradbury *et al.* 2007). The table
166 grape/raisin genome of Thompson Seedless was used, in addition to the wine grape-derived
167 inbred genome of PN40024, to capture some of the variability in SNP detection between
168 reference genomes. Quality (≤ 20) and length (≥ 20 bp) filtered and trimmed reads were aligned
169 to the Thompson Seedless genome (Genova *et al.* 2014; Patel *et al.* 2018) and the PN40024
170 12xv2 genome (Canaguier et al., 2017) using BWA (Li and Durbin 2010). Identified SNPs were
171 further filtered for frequency of minor (0.20) and major (≥ 0.35) allele frequencies, missing data
172 ($\leq 10\%$), and sequencing depth (≥ 5 reads) using vcftools 0.1.15 (Danecek *et al.* 2011). For the
173 PN40024 genome, an additional filtering step to thin SNPs based on physical position to a
174 minimum of 50 bp between sites was completed. A panel of 10,122 and 3,997 filtered SNPs
175 (Thompson Seedless and PN40024, respectively) were used for genome-wide association
176 analyses for each of the ions measured (Table 1). A kinship matrix was estimated in Tassel v
177 5.2.43 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 principal 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) $\leq$ 0.05. The continuity of the Thompson Seedless genome is of lower quality than that of PN40024. Thus to cross reference significant SNPs detected in each reference genome, PN40024 was used as a coordinate reference. GBS tags with significant SNPs detected in Thompson seedless and their associated flanking sequence were mapped back to PN40024 to unify coordinates. Sequences were mapped using default parameters for short read alignment using Minimap2 (Li 2018). Uniquely mapped primary alignments with quality higher than 40 were kept in the lift-over. Manhattan plots for chromosome 3 were produced using the CMplot package in R (https://github.com/YinLiLin/R-CMplot).This method allowed for the ordering of SNPs in the same coordinate reference and comparison between distributions.

Functional annotation of genes associated with SNPs was determined using Blast2GO v 5.2.5 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 2). Large increases in Al, Fe, Mg, Zn, Mn and Ca concentrations were observed in leaf tissue between samples collected in 2015 and 2016. Mean values for nutrient concentrations varied for most of the ions evaluated among individuals in the VxT population (Supplemental Figure 1, Supplemental Table 1). 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 3). Higher levels of Mg, Na, Al, and Fe were observed in symptomatic plants in both years, while a decrease in N was observed. Na, P, Cu, Mn, N, S, and Ca concentrations in 2015 or 2016 had no calculable heritability. 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 (Figure 2, Table 4, Supplemental Figure 2). In 2015 Mg concentrations ($r = 0.6146$) and in 2016 Al concentrations ($r = 0.4805$) had the highest correlation with observed vineyard symptoms (Table 4). Na and Fe concentrations were also correlated with symptoms in both years, though at lower r values. In 2015, a significant negative correlation between S concentration (22%) and symptoms was observed, but not in 2016 (Supplemental Table 2). In 2016, there was a significant negative correlation between P, Cu, and K content and symptoms (Supplemental Table 2). Other significant correlations among nutrients included positive correlations ($r \geq 0.30$) between N, P, and S in 2015 and S, P, and K in 2015 and 2016 (Supplemental Table 2). A strong positive correlation was also detected for both Mn and Ca with Mg in 2015, but not 2016. Correlation with symptoms were observed for other nutrients, but were not consistent between years. When comparing nutrient concentrations from 2015 to 2016, most nutrients had low to moderate (r =

0.2 – 0.4) correlation, with the exception of copper (r= - 0.0199) (Supplemental Table 3). Nutrient ratios were examined between years for potential significant correlations with symptoms. Most ratios did not show consistent differences in values between years for symptomatic and non symptomatic vines (Supplemental Table 4).

Marker-trait associations

Genome-wide associations identified several chromosomes associated with differences in the ions evaluated (Supplemental Figure 3, Figure 3). Significant positive associations between SNPs on chromosome 3 and Mg concentration were detected in 2015 for both the Thompson Seedless and PN40024 genomes. SNPs associated with Mg levels explained approximately 6% of trait variation (Supplemental Table 5). In 2015, 6 SNPs were detected when aligned to the PN40024 genome while 4 SNPs were detected when aligned to Thompson Seedless. No SNPs associated with Mg accumulation were identified in 2016 with either genome at the $P = 0.05$ FDR level. Only 1 genic SNPs associated with Mg concentration was identified and was co-associated with SNPs identified for marginal leaf chlorosis and stunting (Supplemental Tables 6 and 7). In the Thompson Seedless genome, a small block of SNPs (S3_21825918 , S3_21825925, and S3_21825966), spanning 48 bp, located in a ~ 7,000 bp intra genomic region, explained ~ 18% of the variation associated with symptoms in 2015. A BLAST search of the region did not identify any significant alignments with any genes (predicted, putative or known) in *Vitis* or other species.

SNPs associated with Na concentration were detected on chromosomes 11, 12, 13, and 18 had both negative and positive allelic effects ranging from 8 to 13% of the observed variation in the Thompson Seedless genome. Three of the SNPs (S11_16152632, S18_25745134, and S18_25745143) were detected in 2015 and 2016. None of the SNPs associated with Na concentration in 2015 were located in predicted or known genes. Only a single SNP identified in 2016 (S11_11635675) was found in an annotated gene (Supplemental Table 6). In the PN40024 genome, 11 SNPs with positive allelic effects 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 adjusted for an FDR of 0.05. Using the PN40024 genome annotation,

8 genes were associated with Na accumulation. These included genes putatively involved metabolism and transport. Four of the genes had no functional annotation ascribed (Table 5). One gene, Vitvi11g01139, was associated with Na accumulation and annotated as a Clathrin assembly protein, which is a class of proteins involved in macromolecule transportation. No significant SNPs were identified for any of the remaining ions measured.

For marginal chlorosis, a total of 151 SNPs, 57 in 2015 and 94 in 2016 were detected across 2015 and 2016, respectively in the Thompson Seedless genome. In 2015, 33 of the identified SNPs were located in genes, while in 2016, 20 SNPs were located in genes (Table 6). Sixteen of the genic SNPs were shared between years. Individual SNP effects on the Thompson Seedless genome were both negative and positive and ranged from 16 to 33%. Many of the SNPs identified in only a single year were located in genes with multiple SNPs associated with the trait (Supplemental Table 6). When aligned to the PN40024 genome, a total of 29 and 55 SNPs in 2015 and 2016, respectively associated with marginal chlorosis were detected. Marginal chlorosis was primarily associated with SNPs on chromosome 3 with other SNPs detected on chromosomes 6, 9, 13, 16, 17 and 19. In 2015, 23 of the identified SNPs were located in genes, while in 2016, 22 SNPs were located in genes, 22 SNPs were shared between years. Similar to the SNPs detected in Thompson Seedless genome, many SNPs were consistent across both years on chromosomes 3 and 16.

A total of 37 and 71 SNPs associated with stunting were identified in 2015 and 2016, respectively when mapped to the Thompson Seedless genome. SNP effects varied from 3 up to 40%. Highest effect SNPs were detected on chromosome 3 with smaller effect SNPs located on chromosomes 18 (2015) and 1, 2, 10, 11, 12, 16 and 18 (2016). Twenty-four and 34 SNPs were associated with genes in 2015 and 2016, respectively. Nineteen of the genic SNPs associated with plant stunting were shared between years. The majority of single-year SNPs were identified in 2016 (Supplemental Table 5). When mapped to the PN40024 genome, a total of 34 and 44 SNPs were detected in 2015 and 2016, respectively. Only 34 and 35 SNPs associated with stunting were located in genes for 2015 and 2016, respectively of which 29 were shared between 2015 and 2016.

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 4). 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 6). When symptom-associated SNPs located in genes based on the PN40024 annotation were evaluated for putative activity, stress response, transcription, growth and development, and metabolic pathways were all represented similar to the Thompson Seedless 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).

The majority of symptom-associated SNPs were found on chromosome 3 in both genomes, but each genome has a unique coordinate system. Therefore, we performed a consolidated genome analysis by mapping significant SNPs in Thompson seedless and their flanking regions to the PN40024 genome in order to order SNP coordinates and look for overlap between references (Figure 4). When chromosome 3 assemblies were consolidated, a shared cluster of SNPs with significant association with symptoms was observed ~7.5 Mb and a lesser cluster around ~15Mb. When aligned to the Thompson Seedless genome, symptom-associated SNPs located within genes shared across years were predominantly found on chromosome 3 with an additional SNP located on chromosome 10 (Table 6, Supplemental Table 6). Significant single year SNPs, including those associated in genes, were identified on chromosomes 1, 2, 3, 4, 10, 11, 12, 16, and 18 (Supplemental Table 5). When symptoms were combined, 28 SNPs were shared across both years, and 29 were only identified in a single year (Supplemental Table 5). When aligned to the PN40024 genome, significant SNPs were detected on chromosomes 3, 6, 11, 16, and 19. Most of the identified SNPs were located on chromosome 3, and 21 were shared between 2015 and 2016 (Table 5). Twenty 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. Sixteen genic SNPs associated with the combined symptoms were identified in both 2015 and 2016 (Table 5).

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. We chose to utilize a GWAS style approach to detect significantly associated SNPs amongst the progeny, similar to work by Zou et al. demonstrating genome-wide marker association with flower sex (2020) rather than pursue a traditional QTL approach. This approach allowed us to evaluate the genetic architecture of leaf symptoms through the high SNP detection produced by using next-generation GBS methods. In the case of Na, this approach enabled the detection of significant SNPs, even when low or no heritability was calculated, likely due to the low Na concentrations observed (0.0 to 0.2 % dm).

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). This difficulty is exemplified by the results presented here, where significant changes in ion concentrations varied in the two years of the study. Most ions showed an increase in concentration from 2015 to 2016, with the exception of B, P, N, K despite higher levels being applied in 2016. However, low correlations between years indicated that year x genotype played a substantial role in ion concentrations. These higher levels of ions in 2016, likely contributed to the increased number of SNPs detected in 2016 compared to 2015. In the VxT population, P, B, and Cu concentrations were within previously reported "normal" limits for *V. vinifera* petioles (Bates and Wolf 2008), had no correlation with observed physiological symptoms, and low to moderate variability among individuals. 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 a small 48 bp block of 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). 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 (Henderson et al., 2017) in addition to chromosomes 3 and 18. Henderson et al. (2017) and Wu et al. (2020) found variability in high affinity potassium transporters (HKT) that could improve exclusion of Na in grape leaves, using interspecific hybrids from *V. champinii* and *V. rupestris* and later in *V. vinifera*. The SNP identified on chromosome 11 and found in 2015 and 2016 in the PN40024 genome did not co-localize to regions with known *Vvi*HKT members, and may be a novel modifier of leaf Na exclusion. In our study, individual SNP (genic and non-genic) effects varied widely. 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.

Grape has a high level of heterozygosity, and separating genotype errors from minor alleles can be challenging (Hyma et al., 2015). 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 PN40024 genomes. Additionally, as 'Gewürztraminer' is an aromatic sport of 'Traminer', which itself is the parent of 'Verdejo', this population is genetically similar to an F_1 back cross 1 (F_1BC_1). The apparent presentation of symptomatic vines in a 3:1 recessive pattern also suggests that both parents may carry associated genes in a heterozygous state that when combined, produce the undesirable trait. 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. While speculative, it is possible that the wide distribution of many SNPs of varying effects across chromosome 3 suggests this chromosome may by carrying deleterious alleles. While multiple SNPs were identified in this study, additional work is needed to confirm their role in nutrient accumulation. 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 high variability in effects on symptoms (leaf stunting and/or chlorosis), with few genes having more than one significant SNP. In the Thompson Seedless genome annotation, most Na associated SNPs were located in large intergenic regions of the genome. Fewer significant SNPs were detected in the PN40024 genome compared to the Thompson Seedless, as was expected due to the increased filtering in the PN40024 genome. However, in Thompson Seedless, multiple SNPs within a single gene were detected, but not for the PN40024 genome suggesting that higher stringencies of filtering could make the dataset more manageable without losing too many regions of interest. The combination of a low read depth threshold and the absence of genetic mapping could result in genotyping errors, which may be a source of error. These data highlight the importance of genome, annotation and filtering, selection when performing these types of studies.

Conclusion

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

and showed no variability in the population for copper concentration. For nutrients with high variability in the population, this low broad sense heritability is indicative of a large environmental component. This was further evident in that specific nutrient concentrations fluctuated with environmental conditions, vine age or the interaction between environment and individual genotype 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, this bi-parental population had insufficient recombination in the region to identify associated candidate genes. 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. We would like to thank Dr. Konstantin Divilov for assistance with testing BLUPs. 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.

490 Baneh HD, Hassani A, Shaieste FG. 2014. Effects of salinity on leaf mineral composition and
491 salt injury symptoms of some Iranian wild grapevine (*Vitis vinifera* L. ssp. *sylvestris*)
492 genotypes. J Int Sci Wine and Vine 48:1692.
493
494 Bates T, Wolf T. 2008. Vineyard Nutrient Management. Wolf T. (eds) In Wine Grape
495 Production for Eastern North America. NRAES, Ithaca NY
496
497 Benito A, Romero I, Dominguez N, Garcia-Escudero E, Martin I. 2013. Leaf blad and petiole
498 analysis for nutrient diagnosis in *Vitis vinifera* L. cv. Garnacha tinta. Aus j Grape Wine Res
499 19:285-298.
500
501 Bernstein L. 1975. Effects of salinity and sodicity on plant growth. Ann. Rev. Phytopath.
502 13:295-312.
503
504 Bert PF, Bordenave L, Donnart M, Hevin C, Ollat N, Decroocq S. 2016. Mapping genetic loci
505 for tolerance to lime-induced iron deficiency chlorosis in grapevine rootstocks (*Vitis* sp.)
506 Theo Appl Genet 126:451-473.
507
508 Black CA. 1965. Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, Number
509 9, 2nd Ed. American Society of Agronomy (ASA) Madison, WI.
510
511 Bose J, Babourina O, Rengel Z. 2011. Role of magnesium in alleviation of aluminum toxicity in
512 plants. J Exp Bot 63:2251-2264.
513
514 Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. 2007. TASSEL:
515 software for association mapping of complex traits in diverse samples. Bioinformatics
516 23:2633-2635.
517
518 Brancadoro L, Rabotti G, Scienza A, Zocchi G. 1995. Mechansims of Fe-efficiency in roots of
519 *Vitis* spp. in response to iron deficiency stress. Plant and Soil 2:229-234.
520
521 Canaguier A, Grimplet J, Di Gaspero G, Scalabrin S, Duchene E, Choisne N, Mohellibi N,
522 Guichard C, Rombauts S, Le Clainche I, Berard A, Chauveau A, Bounon R, Rustenholz C,
523 MOrgante M, Le Paslier MC, Brunel D, Adam-Blondon AF. 2017. A new version of the
524 grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). Genom
525 Data 14:56-62.
526
527 Cancado GMA, Ribeiro AP, Pineros MA, Miyata LY, Alvarenga AA, Villa F, Pasqual M,
528 Purgatto E. 2009. Evaluation of aluminium tolerance in grapevine rootstocks. Vitis 48:167-
529 173.
530
531 Carbonell-Barrachina AA, Burlo-Carbonell F, Mataix-Beneyto J. 2008. Effect of sodium
532 arsenite and sodium chloride on bean plant nutrition (macronutrients). 20:1617-1633.
533
534 Christensen P. 1984. Nutrient level comparisons of leaf petioles and blades in twenty-six grape
535 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.

Conradie WJ. 1992. Partitioning of nitrogen by the grapevine during autumn and the utilization of nitrogen reserves during the following growing season. S African J Enol Vit 13:45-51.

Conradie WJ, Saayman D. 1989. Effects of long-term nitrogen, phosphorus, and potassium fertilization on Chenin Blanc vines. II. Leaf analyses and grape composition. Amer J Enol Vit 40:91-98.

Cordovilla MP, Ocana A, Libero F, Lluch C. 1995. Salinity effects on growth analysis and nutrient composition in four grain legumes – rhizobium symbiosis. J Plant Nut 18:1595 – 1609.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker R, Lunter G, Marth G, Sherry ST, McVean G, Durbin R, 1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. Bioinformatics. 27:2156-2158.

Davies C, Shin R, Liu W, Thomas MR, Schachtman DP. 2006. Transporters expressed during grape berry (*Vitis vinifera* L.) development are associated with an increase in berry size and berry potassium accumulation. J Exp Bot 57:3209-3216.

Downton WJS. 1977. Chloride accumulation in different species of grape-vine. Sci Hort 7:249-253.

Fehr WR. 1987. Principles of cultivar development. Macmillan, Inc. New York.

Genova AD, Almeida AM, Munoz-Espinoza C, Vizoso P, Travisany D, Moraga C, Pinto M, Hinrichsen P, Orellana A, Maass A. 2014. Whole genome comparison between table and wine grapes reveals a comprehensive catalog of structural variants. BMC Plant Biology 14:7.

Gong HJ, Blackmore DH, Clingeleffer PR, Sykes SR, Walker RR. 2015. Variation for potassium and sodium accumulation in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri. J Grape Res 53:65-72.

Gotz S, Garcia-Gomez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talon M, Dopazo J, Conesa A. 2008. High-throughput functional annotation and data mining with the Blast2GO suite. Nucl Acid Res 36:3420-3435.

Grattan SR, Grieve CM. 1992. Mineral element acquisition and growth response of plants grown in saline environments. Ag Eco Env 38:275-300.

Guo W, Nazim H, Liang Z, Yang D. 2016. Magnesium deficiency in plants: An urgent problem. Crop Journal 4:83-91.

Henderson CR. 1975. Best linear unbiased estimation and prediction under a selection model. Biometrics 31:423-447.

Henderson SW, Dunlevy JD, Wu Y, Blackmore DH, Walker RR, Edwards EJ, Gilliham M, Walker AR. 2017. Functional differences in transport properties of natural HKT1;1 variants influence shoot Na+ exclusion in grapevine rootstocks. New Phyto 217:1113-1127.

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.

Hyma KE, Barba P, Wang M, Londo JP, Acharya CB, Mitchell SE, Sun Q, Reisch B, Cadle-Davidson L. 2015. Heterozygous mapping strategy (HetMappS) for high resolution genotyping-by-sequencing markers: A case study in grapevine. PlosOne e0134880

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]

Li H. 2018. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*, **34**:3094-3100. doi:10.1093/bioinformatics/bty191

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.

Liu XQ, Rong JY, Liu XY. 2008. Best linear unbiased prediction for linear combinations in general mixed linear models. J of Multivariate Analysis 99:1503-1517.

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.

Merk HL. 2011. Estimating heritability and BLUPs for traits using tomato phenotypic data. https://plant-breeding-genomics.extension.org/estimating-heritability-and-blups-for-traits-using-tomato-phenotypic-data/

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.

672 Patel S, Lu Z, Jin X, Swaminathan P, Zeng E, Fennell AY. 2018. Comparison of three assembly
673 strategies for a heterozygous seedless grape genome assembly. BMC Genomics 19:57.
674
675 Perez-Castro R, Kasai K, Gainza-Cortes F, Ruiz-Lara S, Casaretto JA, Pena-Cortes H, Tapia J,
676 Fujiwara T, Gonzalez E. 2012. *VvBOR1*, the grapevine ortholog of *AtBOR1*, encodes an efflux
677 boron transporter that is differentially expressed throughout reproductive development of
678 *Vitis vinifera* L. Plant and Cell Phys. 53:485-494.
679
680 Poor D, Pedo S, Bertoldi D, Bortolotti L, Failla O, Zamboni M. 2013. Evaluation of new
681 rootstocks for grapevine: nutritional aspects. Proc VIIth IS Min. Nut. Fruit Crop Eds.:
682 Poovarodom and Yingjajaval S. Acta Hort. 984, ISHS.
683
684 Pradubsuk S, Davenport JR. 2010. Seasonal uptake and partitioning of macronutrients in
685 mature 'Concord' grape. J Amer Soc Hort 135:474-483.
686
687 Primikiros NI, Roubelakis-Angelakis KA. 2001. Indications for post-translational regulation
688 of *Vitis vinifera* L., arginine decarboxylase. Plant Molec Biol 45:669-678.
689
690 R Core Development Team. 2017. R Foundation for Statistical Computing Vienna, Austria.
691 https://www.R-project.org/
692
693 Rashad RT, Hussein RA. 2014. A comparison study on the effect of some growth regulators
694 on the nutrients content of maize plant under salinity conditions. Ann Ag Sci 59:89-94.
695
696 Rogier SY, Keller M, Holzapfel BP, Virgona JM. 2000. Accumulation of potassium and
697 calcium by ripening berries on field vines of *Vitis vinifera* (L) cv. Shiraz. Aus J Grape Wine
698 Res 6:240-243.
699
700 Schreiner RP, Scagel CF, Baham J. 2006. Nutrient uptake and distribution in a mature ' noir'
701 vineyard. HortSci 41:336-345.
702
703 Schreiner RP. 2016. Nutrient uptake and distribution in young Pinot noir grapevines over
704 two seasons. Amer Soc. Enol Vit 67:436-448.
705
706 Shani U, Ben-Gal A. 2005. Long-term response of grapevines to salinity: Osmotic effects and
707 ion toxicity. Am J Enol Vitic 56:148-154.
708
709 Shaul O. 2002. Magnesium transport and function in plants: the tip of the iceberg. BioMetals
710 15:309-323.
711
712 Shikhamany SD, Chititrai CR, Chadha KL. 1988. Effect of varying levels of nitrogen and
713 potash on petiole nutrient concents in Thompson Seedless grape (*Vitis vinifera* L.)
714
715 Sinclair C, Hoffman AA. 2003. Monitoring salt stress in grapevines: are measures of plant
716 trait variability useful? J Appl Ecol 40:928-937.
717

718 Skinner PW, Matthews MA. 1990. A novel interaction of magnesium translocation with the
719 supply of phosphorous to roots of grapevine (*Vitis vinifera* L.) Plant Cell Env 13:821-826.
720
721 Spiers JM, Braswell JH. 1994. 793 PB 039 Calcium, magnesium, and nitrogen fertilization
722 affects leaf nutrient content and growth of 'Sterling' muscadine grape. HortSci 29:546.
723
724 Tagliavini M, Romboloa D. 2001. Iron deficiency and chlorosis in orchard and vineyard
725 ecosystems. E J Ag 15:71-92.
726
727 Tang RJ, Zhao FG, Garcia VJ, Kleist TJ, Yang L, Zhang HX, Luan S. 2010. Tonoplast CBL-CIPK
728 calcium signaling network regulates magnesium homeostasis in *Arabidopsis*. PNAS
729 112:3134-3139.
730
731 Tavakkoli E, Rengasamy P, McDonald GK. 2010. High concentrations of Na+ and Cl- ions in
732 soil solution have simultaneous detrimental effects on growth of faba bean under salinity
733 stress. J Exp Bot 61:4449-4459.
734
735 Thenabadu MW. 1968. Magnesium-sodium interactions effecting the uptake and
736 distribution of potassium and calcium by cotton. Plant and Soil 29:132-143.
737
738 Venkatesan S, Jayaganesh S. 2010. Characterization of magnesium toxicity, its influence on
739 amino acid synthesis pathway and biochemical parameters of tea. Res J Phytochem 4:67-
740 77.
741
742 Verbruggen N, Hermans C. 2013. Physiological and molecular responses to magnesium
743 nutritional imbalance in plants. Plant and Soil 368:87-99.
744
745 Volkmar KM, Hu Y, and Steppuhn H. 1998. Physiological responses of plants to salinity: A
746 review. Can J Plant Sci 78:19-27.
747
748 Wang D, Karle R, Iezzoni AF. 2000. QTL analysis of flower and fruit traits in sour cherry.
749 Theor Appl Gent 100:535-544.
750
751 Wilcox WF, Gubler WD, and Uyemoto JK. 2015. Compendium of grape diseases, disorders,
752 and pests. 2nd Ed. Pp.159-167. APS Press St. Paul, MN.
753
754 Williams CMJ, Maier NA, Bartlett L. 2004. Effect of molybdenum foliar sprays on yield, berry
755 size, seed formation, and petiolar nutrient composition of "Merlot" grapevines. J Plant Nut
756 27:1891-1916
757
758 Wu Y, Henderson SW, Wege S, Zheng F, Walker AR, Walker RR, Giliham M. 2020. The
759 grapevine NaE sodium exclusion locus encodes sodium transporters with diverse transport
760 properties and localization. J Plant Phys. 246-247.
761
762 Zhang Z. 2016. GAPIT version 2: an enhanced integrated tool for genomic association and
763 prediction. Plant Genome 9:2

764
765 Zhu JK, Liu J, Xiong L. 1998. Genetic analysis of salt tolerance in Arabidopsis: Evidence for a
766 critical role of potassium nutrition. Plant Cell. 10:1181-1191.
767
768 Zou C, Karn A, Reisch B, Nguyen A, Sun Y, Bao Y, Campbell MS, Church D, Williams S, Xu
769 X, Ledbetter CA, Patel S, Fennell A, Glaubitz JC, Clark M, Ware D, Londo JP, Cadle-davidson
770 L. 2020. Haplotyping the *Vitis* collinear core genome with rhAMPSeq improves marker
771 transferability in a diverse genus. Nature Communications 11:413.
772

Table 1(on next page)

SNP distribution across chromosomes

Table 1. Single Nucleotide Polymorphism distribution across the Thompson Seedless and PN40024 reference genomes.

Chromosome	Number of SNPs	
	Thompson Seedless	**PN40024**
1	608	189
2	271	107
3	385	180
4	543	225
5	746	322
6	479	207
7	663	240
8	640	260
9	476	214
10	676	253
11	279	113
12	624	257
13	609	241
14	753	278
15	436	137
16	418	180
17	463	172
18	655	283
19	398	139
Total	**10,122**	**3,997**

Table 2(on next page)

Mean nutrient concentrations by year

Table 2. 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 3(on next page)

Population mean nutrient concentrations

Table 3. 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 4(on next page)

Pearson correlations of nutrients

1

2 **Table 4**. Correlation (r) among nutrient concentrations in 2015 (gray) and 2016 (white) from
3 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*	-

4 [A] Symptoms

5 *P ≤ 0.05

6 **P < 0.001

7 ***P < 0.0001

8 NS = not significant

Table 5(on next page)

Significant SNPs in genes PN40024

Table 5. Genic Single Nucleotide Polymorphisms (SNPs) associated with symptoms (marginal leaf chlorosis and stunting) using the PN40024 genome annotation and NCBI in 2015 and 2016 in an F_1 population of *V. vinifera*.

Chr[A]	Gene[B]	SNP	Effect[C]	Putative function[D]
3	Vitvi03g00380	S3_4196400	23-25%	Unknown
	Vitvi03g01518	S3_4201002	(-)25%	PREDICTED: uncharacterized protein
	Vitvi03g00384	S3_4208958	21-25%	Integral membrane protein
	Vitvi03g00384	S3_4209015	21-25%	Integral membrane protein
	Vitvi03g00430	S3_4637832	(-)23-27%	Dof zinc finger protein DOF5.8
	Vitvi03g00520	S3_5653914	24-27%	Basic helix-loop-helix (bHLH) family
	Vitvi03g00534	S3_5852953	23-24%	ABA-specific glucosyltransferase
	Vitvi03g00543	S3_5986778	(-)26-30%	DNA-directed RNA polymerase II
	Vitvi03g00560	S3_6167883	29-31%	UNC-50
	Vitvi03g00583	S3_6554413	(-)23%	TIP41
	Vitvi03g00603	S3_6823070	29-32%	R protein MLA10
	Vitvi03g00688	S3_7815436	33%	Hypothetical protein
	Vitvi03g00688	S3_7815488	(-)33%	
	Vitvi03g00777	S3_9374358	25-29%	EMB2758 (embryo defective 2758)
	Vitvi03g01012	S3_14786293	(-)29%	No hit
	Vitvi03g01792	S3_16473090	(-)26-31%	Peru 1

[A] Chromosome

[B] Putative grape gene based on the PN40024 v2 genome (Canaguier et al., 2017).

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

[D] Functional annotation based on PN40024 genome v3 annotation (Canaguier et al., 2017).

Table 6(on next page)

Significant SNPs in genes

Table 6. 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).

Figure 1

Figure 1 - Leaves

Figure 1. (A) Asymptomatic and (B) symptomatic (stunted) grape stem internodes and (C-D) leaves from an F1 V. vinifera population. E. Asymptomatic and symptomatic (marginal leaf chlorosis and stunted) leaves.

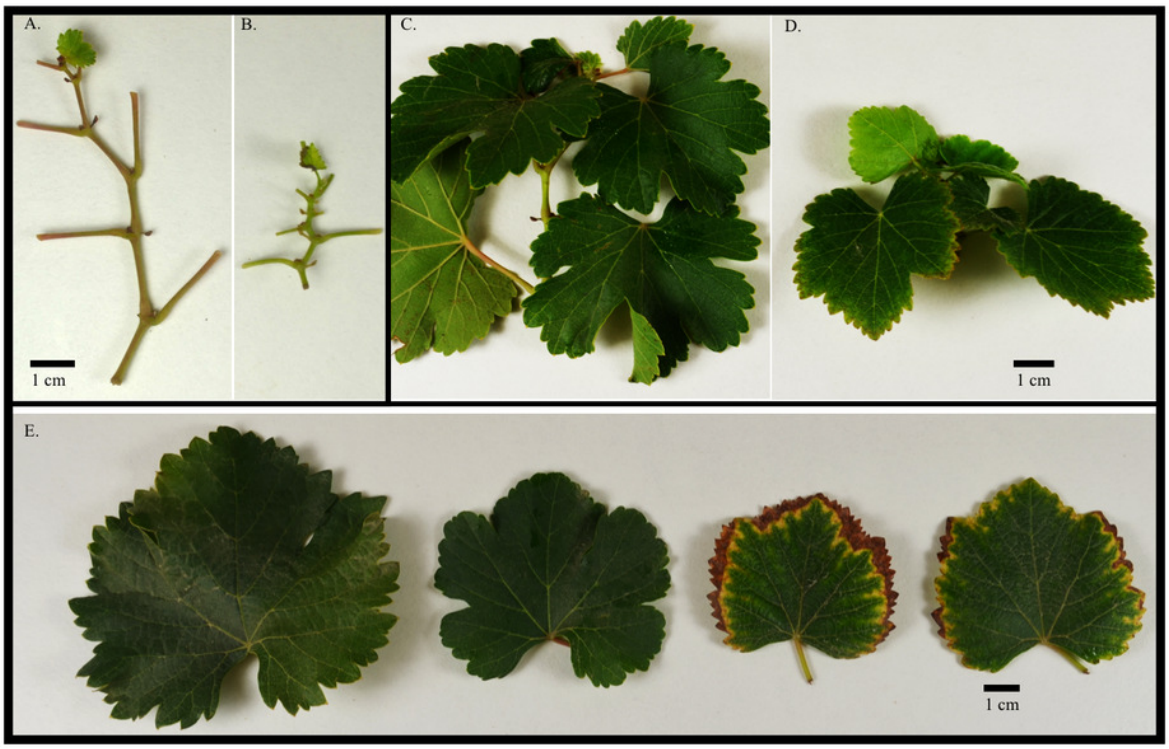

Figure 2

Figure 2 - PCA

Figure 2. Principal Component Analysis (PCA) based on mean Best Linear Unbiased Predictors (BLUPs) for nutrient concentrations of magnesium (Mg), sodium (Na), aluminum (Al), iron (Fe), potassium (K), phosphorous (P), nitrogen (N), sulfur (S), zinc (Zn), boron (B), and calcium (Ca). A. Black circles represent non symptomatic plants, blue diamonds indicate plants that exhibited symptoms (only marginal leaf chlorosis (MC) or stunting (SL)) in only one year, teal triangles indicate plants that had symptoms (MC and SL) in only one year, black asterisks represent plants that had SL or MC for both years, and pink squares indicate vines with stunting and leaf chlorosis in both years. B. Vectors for each nutrient based on BLUPs.

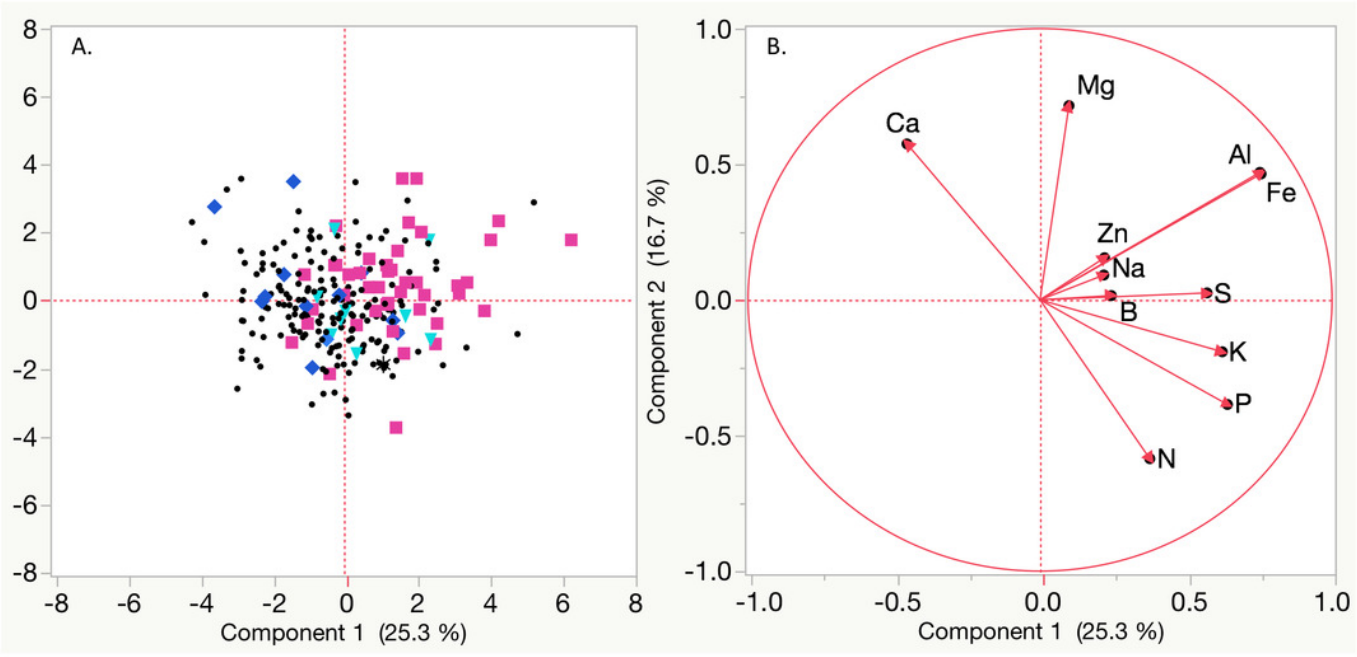

Figure 3

Figure 3 - manhattan plot

Figure 3. Manhattan plot of Single Nucleotide Polymorphisms (SNPs) associated with marginal leaf chlorosis/burn and stunting symptoms in a *V. vinifera* F_1 segregating population in A. 2015 and B. 2016 aligned to the PN40024 genome. The green horizontal line denotes the genome-wide significance threshold at $P < 1.0 \times 10^{-7}$.

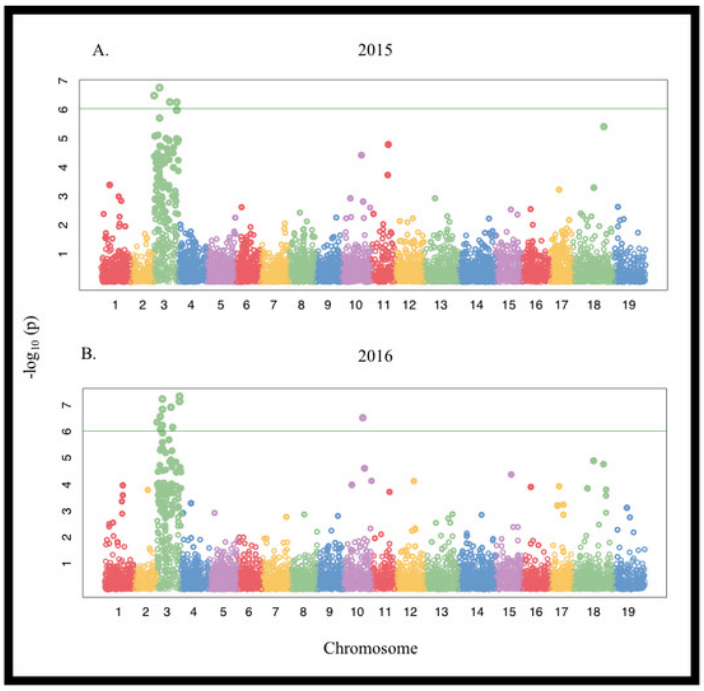

Figure 4

Fig4 - chromosome structure comparison

Figure 4. Coordinate comparison of the distribution of non significant and significant SNPs identified on chromosome 3 between Thompson Seedless (TS) and PN400424 (PN) reference genomes. The dotted line denotes significance at $P = 0.05$.