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# LEAF NUTRIENT STATUS OF 'TRAKYA İLKEREN' GRAPE VARIETY (Vitis vinifera L.) IN DIFFERENT PHENOLOGICAL STAGES

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### ABSTRACT

Nutrition of grapevines is very sensitive issue in vineyards. Soil quality is very important matter for growth and quality in vine growing. Rootstocks affect the growth and productivity of grapevine as well as increase or decrease of the nutrient uptake. The present study was conducted to determine the phenological changes of nutrient uptakes in 10 years 'Trakya İlkeren' grape variety (Vitis vinifera L.) in the heavy clay soil conditions. The grapevines are grown on 5BB and 5C rootstocks. The changes of macro and micronutrients in leaf blades from bud burst to post harvest period were investigated in the experiment. Leaf nutrient contents of leaf blades show varied depending on the phenological stages and rootstocks (P < 0.01 and P < 0.05). Nitrogen and phosphorus content of leaf blade was decreased until veraison stage for both rootstocks. The highest potassium (K) content was obtained at blooming stage. In blooming stage nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg) nutrients was found high on 5C rootstock whereas calcium (Ca) was high on 5BB. Grapevines were found insufficient for P, K and Mg nutrients in the study. Total chlorophyll and chlorophyll a/b ratio showed significantly varied among to rootstocks and phenological stages (P < 0.01). While the highest leaf chlorophyll content was measured during flowering period it was found at the lowest through to harvest on both rootstocks. Overall total chlorophyll contents and chlorophyll *a/b* ratio were significantly higher on 5BB grafted vines. In the research, 5C was found to be more successful rootstock than 5BB for nutrient uptakes.

Key words: grapevine, leaf nutrients, chlorophyll, rootstocks, phenology

# INTRODUCTION

The effect of the rootstock on the nutrition of the grapevines is known for a long time. Rootstocks affect the vigour of shoot growth, productivity of grapevine, increase or decrease of the nutrient uptake as well as except for phylloxera resistant [Boselli et al. 1992, Ferroni and Scalabrelli 1995, Verma et al. 2010]. Grapevine is a perennial crop in which mineral nutrient concentration changes during the growing season [Pradubsuk and Davenport 2010]. Rootstocks and plant nutritional status influence mineral uptake and thus fruit yield and quality [Grant and Matthews 1996, Bavaresco et al. 2003, Nikolaou et al. 2003]. Rootstocks have also significant impacts on mineral uptake of grapevines [Keller et al. 2001]. It is already known that the rootstock affects grape leaf mineral contents, especially K and Mg [Dalbó et al. 2011]. Smart et al. [2006] carried out at over 200 root studies and they found



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that average 63% of roots were found in the top 60 cm of soil. With the ecological factors, soil parameters have a most influence on root growth and distribution. Soil texture influences the rooting depth and vertical distribution of roots [Nagarajah 1987]. Fertilization of grapevines depends on the rootstocks, which is differing in their uptake of macro and micronutrients, as well as on the vineyard soils' physical and chemical characteristics [Lambert et al. 2008]. Plants need sufficient macro and micronutrients to complete physiological and biochemical functions [Balasubrahmanyam et al. 1978, Shaaban and El-Fouly 2012, Brataševec et al. 2013]. Also, basic minerals (N, P, K) and some other elements (Mg, Fe, Zn, B etc.) plays important roles in various metabolic enzyme activities and processes [Bergmann 1992, Marschner 1995]. N levels of grapevines generally decreases between the periods of blooming and veraison [Csikász-Krizsics and Diófási 2008]. Vercesi [1987] stated that mineral uptake abilities of the rootstocks based on genetic characteristics.

The aim of the study is to determine the mineral uptakes in different phenological stages of the 'Trakya İlkeren' grapevine variety (*Vitis vinifera* L.) which was grafted on 5BB and 5C rootstocks on heavy clay soil conditions.

# MATERIALS AND METHODS

The experiment was conducted in the experimental vineyard of the Ondokuz Mayıs University Agricultural Faculty in 2013 growth season. The study area is located at 41°21'52N latitude and 36°11'29E longitude, at an altitude of 195 m and a distance of approximately 2.8 km from the Black Sea coast, Turkey. In the study, 'Trakya İlkeren' grape variety (Alphonse Lavallée × Perlette) was used. This grape has a black skin and early ripening table grape. It is suitable as a wrapping leaf for foods and under greenhouse cultivation. The grapevines were 10 years old, and grafted on to 5BB and 5C rootstocks. They were also trained onto a double cordon system with  $3 \times 1.5$  m spacing. Grapevines were not irrigated, and the only supplementary water they received was from rainfall. They were also not fertilized. Experiment area had clay soil type. Physical characteristics of experimental vineyard are provided in Table 1. In the study, leaf nutrient contents of grapevines were evaluated according to Zengin 2012 (tab. 2).

**Table 1.** Physical properties and nutrient contents of the vineyard soil

Soil pro	operties	Nutrient element contents				
Clay (%)	62.12	Total N (g·kg <sup>-1</sup> )	2.12			
Silt (%)	20.09	$P(g \cdot kg^{-1})$	4.27			
Sand (%)	17.79	K (meq·100 g <sup>-1</sup> )	1.07			
Texture class	$\mathbf{C}^*$	Ca (meq·100 g <sup>-1</sup> )	28.39			
pH, sat. Ext.	7.1	Mg (meq $\cdot 100 \text{ g}^{-1}$ )	8.96			
EC ( $dS \cdot m^{-1}$ )	0.69	Fe (mg·kg <sup>-1</sup> )	12.02			
OM (%)	4.33	Mn (mg·kg <sup>-1</sup> )	23.63			
Lime (CaCO <sub>3</sub> ), (%)	0.85	Zn (mg·kg <sup>-1</sup> )	1.82			
CEC (meq·100 g <sup>-1</sup> )	38.85	Cu (mg·kg <sup>-1</sup> )	1.11			

\* Clay

Nutrients	Insufficient	Adequate	High	
$N (g \cdot kg^{-1})$	15.0–19.9	20.0-23.0	>24.0	
$P(g \cdot kg^{-1})$	2.2–2.9	3.0-4.0	>4.0	
$K (g \cdot kg^{-1})$	10-12.9	13–14	>14	
Ca $(g \cdot kg^{-1})$	15–19.9	20-25	>25	
Mg $(g \cdot kg^{-1})$	2–2.4	2.5–5	>5	
Fe (mg·kg <sup>-1</sup> )	50–59	60–175	>175	
Zn (mg·kg <sup>-1</sup> )	18–24	25-100	>100	
Mn (mg·kg <sup>-1</sup> )	25–29	30-300	>300	
B (mg·kg <sup>-1</sup> )	20–24	25–75	>70	
Cu (mg·kg <sup>-1</sup> )	3.00-4.00	5–50	>50	

Table 2. Leaf blade nutrient contents in blooming to veraison stage for grapevine [Zengin 2012]

**Chlorophyll analysis.** Chlorophyll *a* (Ch<sub>*a*</sub>) and hlorophyll *b* (Ch<sub>*b*</sub>) concentrations were determined using the method described by Lichtenthaler and Wellburn [1983]. Fresh leaves (0.1 g) were placed into 8 ml of 80% acetone, and filtered through Whatman No. 2 filter paper. Absorbance was measured in a UV-visible spectrophotometer at 646 nm, 663 nm and 470 nm. The Ch<sub>a</sub>, Ch<sub>b</sub> and total chlorophyll ( $\mu$ g·ml<sup>-1</sup> FW) were calculated according to the following equations.

Chlorophyll <sub>a</sub> (Ch<sub>a</sub>) = 12.25 A<sub>663</sub> – 2.798 A<sub>646</sub> Chlorophyll <sub>b</sub> (Ch<sub>b</sub>) = 21.5 A<sub>646</sub> – 5.1 A<sub>663</sub> Total chlorophyll = Ch<sub>a</sub> + Ch<sub>b</sub>

Leaf blades nutrient analysis. In the study, nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulphate (SO<sub>4</sub>), manganese (Mn), copper (Cu), zinc (Zn), iron (Fe) macro and micronutrients were investigated in the grape leaves. Five leaves per shoots were collected to determinate plant nutrient contents during the periods at before blooming, blooming, veraison, harvest and postharvest. Three shoots per vine sampled and leaf samples at the opposite side of a bunch were collected. The leaf blade was immediately separated from the petiole and dried in an oven at 65°C for 48 hours. The dried samples were ground in a hand mill for analysis. The analysis for P concentration was performed by a spectrophotometer after digestion [Barton 1948]. Nitrogen content of leaves was measured by Kjeldahl method [Schuman et al. 1973]. K, Ca, Mg, Na, Fe, Mn, Zn and Cu contents were determined according to the wet digestion method (4 : 1 HNO<sub>3</sub> : HClO<sub>4</sub>) in atomic absorption spectroscopy (Perkin Elmer AA-200) according to Kaçar [1984] and Kaçar and İnal [2008].

Statistical analysis. Complete randomized block design with 3 replication were used in the study. Leaf nutrient contents were measured from four uniformly grown grafted vines per rootstock, and the mean measurements were calculated. Three shoots per vine slected and five leaves per shoots were sampled. The data were analyzed by two-way analysis of variance (ANOVA) to test for interactions between phenological stages and rootstocks. Data analysis was performed using SPSS 16.0 for Windows. Results studied are presented as means and a pooled standard error of mean (SEM). Differences among means were detected using Duncan's multiple range tests at significance levels of (p < 0.01; p < 0.05).

#### **RESULT AND DISCUSSION**

Some physicochemical properties of the vineyard soil to experimental area are given in Table 1. The changes of the leaf macro and micronutrients of 'Trakya İlkeren' grape varieties are shown in Table 3

and Figure 1. The nitrogen (N), phosporus (P) and potassium (K) levels of the grapevine leaves were determined at the highest level before blooming. The N and P content of the leaves on both rootstocks had shown decreased from pre-blooming to veraison stage. In the experiment, although having detected a minimum amount of N during the veraison period at the leaves, it was found adequate N level (tab. 3 and fig.1). Since vines shows very rapid shoot growth in the spring, they needs considerable amount of N from bud burst to early stages of fruit development. Researchers determined that half of the annual N needs to vine growth to be used in this period [Conradie 2005], and then during the growing season it has been found to decrease of using of N [Williams and Biscay 1991]. Therefore, Chen and Cheng [2003] reported that limited N resulted in decreasing chlorophyll contents. According to optimal N distribution, when the N content decreases in leaf, chlorophyll a/b ratio is expected to increase [Kitajima and Hogan 2003].

The N and P content of the leaves on both rootstocks show decreased from pre blooming to veraison season. Bell and Francis [2013] reported that application of N in the vineyard early in the season increased some other elements in the petioles such as Mn and Mg, although N application decreased petiole phosphorus. Indeed, it was reported that potassium uptake show varied according to the phenological stages [Conradie 1981] and of rootstocks [Ruhl 1989, Williams and Smith 1991, Brancadoro et al. 1994]. N and P content of the leaves during the veraison were recorded again increase (fig. 1). The P content of the leaves measured a minimum in the veraison. The phosphorus is a nutrient that plays an important role in determining the nutritional requirements of vines. In the blooming to veraison period the nutrient content of vines P and K values obtained from the experiment showed that a low level compared to a reference value (tab. 2).

 Table 3. Phenological changes of leaf blade macro and micronutrient contents of 'Trakya İlkeren' grape grafted on 5BB and 5C rootstocks

Phenological stages	Rootstocks	Ν	Р	Κ	Mg	Ca	$SO_4$	Mn	Cu	Zn	Fe
			g·kg <sup>-1</sup>					mg⋅kg <sup>-1</sup>			
Pre-blooming	5C	26.4a	2.0b	7.8b	1.52f	6.0e	2.04a	63.40d	18.50a	22.20e	101.45b
	5BB	25.8ab	1.8c	6.9c	2.25c	28.1b	1.50c	62.60d	12.80c	20.55e	77.85cd
Blooming	5C	23.0cd	1.2d	8.7a	1.6f	8.3de	0.95h	43.45f	14.70b	33.10b	71.35d
	5BB	22.3cd	1.1e	6.7c	1.7e	12.0d	0.96h	42.55f	12.60c	24.70d	83.60c
Veraison	5C	21.8de	0.92g	4.9d	2.8b	19.9c	1.19fg	64.55d	9.80d	35.50a	102.20ab
	5BB	21.2e	1.03ef	5.6d	1.95d	22.6c	1.34de	54.75e	6.30e	34.70ab	99.30b
Harvest	5C	23.5c	1.74c	5.3d	3.0a	26.7b	1.78b	98.50a	7.40e	24.55d	77.25cd
	5BB	23.2c	1.0fg	5.0d	1.4g	22.2c	1.41cd	54.42e	3.80f	16.95f	72.75d
Post-harvest	5C	25.3ab	2.3a	4.9d	3.1a	36.4a	1.3ef	87.40b	4.40f	28.10c	109.65a
	5BB	25.0b	2.26a	4.1e	2.0d	35.1a	1.09g	73.15c	4.20f	24.80d	101.05b
Means	5C	24.0	1.6	6.3	2.4	19.5	1.5	71.46	10.96	28.69	102.38
	5BB	23.5	1.4	5.7	1.8	26.0	1.3	60.52	7.94	24.34	86.91
SEM		0.131	0.008	0.076	0.010	0.439	0.008	0.574	0.132	0.204	0.811
Phenological stages **		**	**	**	*	**	**	**	**	**	**
Rootstocks		*	**	**	**	**	**	**	**	**	**

SEM - the standard error of the means. \* P < 0.05, \*\*P < 0.01, ns. non-significant

According to results, before the bud burst period in the spring it should be say that there is a need to fertilization N, P and K with soil application (tab. 3 and fig. 1). Conradie [1981] stated that determination of phosphorus uptake time is very important in terms of determining the best fertilizing time. Although the presence of potassium content of the leaves before blooming period, the highest levels were determined to be well below the optimum level needed to get by the plant. In the study the effect on the leaf N content of the rootstock were not significant. However, the phosphorus content in the grafted grapevines on 5C rootstock was significantly higher compared to on 5BB grafted grapevines. K content of 5C grafted vines at the pre-blooming and full blooming was detected high according to 5BB grafted vines.

The phenological changes of the grapevines in terms of calcium (Ca), sulphate (SO<sub>4</sub>) and magnesium (Mg) are presented in Figure 1. The Ca content was decrease on 5BB pre-bloom to bloom period even though it was constantly increased on 5C grafted vines all periods. The Ca content was found high on 5BB grafted vines in pre blooming stage, whereas it was determined high at harvest period on 5C grafted vines. Ca content was determined significantly different among rootstocks especially pre blooming and harvest periods. In the study, Ca levels were found insufficient at pre-bloom and bloom stages on 5C grafted vines. However, Ca level was found insufficient on blooming stage in 5BB grafted vines (tab. 3). The sulphate content of vine leaves was significantly higher on 5C grafted vines than on 5BB in pre-blooming. The Sulphate content of vine leaves was significantly higher on 5C than on 5BB grafted vines in pre-blooming. It was increasing S content in veraison and harvest periods from blooming, and showed decreasing again after harvest. Sulphate is a necessary element as a component of enzymes, chlorophyll molecules plant amino acids, proteins and vitamins [Bavaresco et al. 2010]. However, sulfur deficiency is not very common situation, and dust sulfur application of leaves does not

apply except for the control of fungal diseases and soil pH. Mg content of the leaves before blooming was found high on 5BB than on 5C grafted vines. Mg content of the leaves during blooming was found closer levels on both rootstocks. Whereas the Mg content was increased until blooming to end of the growth season on 5C grafted vines, it was draw an erratic graphic on 5BB grafted vines. In this study, significant differences were detected between rootstocks level on leaf magnesium content (tab. 2). Mg content of the 5C grafted vines after harvest was found to be higher than 5BB rootstock. In the study, although the Ca and S levels were generally adequate the Mg level was determined insufficient on both rootstocks (tab. 3). Indeed, the Mg is an effective element on the chlorophyll content of leaves and there is chlorosis when the Mg deficiency occurred [Mullins et al. 1992]. Conradie [1981], the suspension of Mg intake has started 22 days after the bud burst and continued also at blooming and veraison periods. It would be appropriate to make Mg fertilizer before flowering.

Phenological changes of some micronutrient contents of the leaves are presented in Figure 1. The micronutrients manganese, copper, zinc and iron (Mn, Cu, Zn, Fe) of grapevines showed significantly varied according to phenological stages and rootstocks (P < 0.01). The leaf Mn content was increase in the between blooming to harvest period on 5C grafted vines, while it was declined during the harvest on 5BB. In particular, the amount of Mn in the harvest period was found higher on 5C grafted vines according to on 5BB grafted. Mn and Fe levels showed a slight decrease from pre blooming to veraison in both root rootstocks.

Fe and Mn are the most important elements in the synthesis of chlorophyll and chlorosis occur in young and mature leaves when deficiency of them [Salisbury and Ross 1992, Bertamini and Nandunchezhian 2005]. In the study, although it was not determined deficiency of Mn at the vine leaves, in 5C grafted vines it was found to be higher than those on 5BB grafted vines (tab. 3 and fig. 1).



Fig. 1. The effect of rootstocks on the leaf blades macro and micronutrient contents of 'Trakya İlkeren' grape grafted on 5BB and 5C rootstocks during phenological stages (phenological P < 0.01, rootstocks P < 0.01)



Fig. 2. Phenological changes of total chlorophyll contents of 'Trakya Ilkeren' grape leaf grafted on 5BB and 5C rootstocks (phenological P < 0.01, rootstocks P < 0.01)



**Fig. 3.** Phenological changes of chlorophyll a/b ratio of 'Trakya Ilkeren' grape leaf grafted on 5BB and 5C rootstocks (phenological P < 0.01, rootstocks P < 0.01)

The Cu content of the leaves before blooming were also higher than other phenological stages in both rootstocks. However, overall the leaf Cu content on 5C grafted vines was determined to be higher than those on 5BB grafted. Zn content of the leaf, in both rootstocks has showed to be erratic during the all phenologic period. While the leaf Zn content was found as maximum in the veraison, it was decreased between veraison and harvest and after it was increased slightly again ends of the growth period. Although Zn content of vine leaves was found a little high on 5C grafted vines, it was determined to be adequate for both rootstocks. Serious problems are observed when emerging Zn deficiency in plants such as reduce fruit-set and decrease the crop load [Shaaban and El-Fouly 2012]. Indeed, when Zn deficiency occurs in young leaves it was chlorosis as well as the leaves are small and short-necked occur [Shange 2006]. In the study, significant differences determined among to rootstock and phenologic stages in terms of the iron content (P < 0.01). The lowest Fe content in leaf blades of the both grafted vines has been identified as the blooming period through to growth season. However, the Fe content of the leaves was found sufficient for vines. Fe is an essential compound in plant for protein and enzyme activity. When the chlorosis occurs due to Fe deficiency in plants, there were decreases photosynthesis activity, vegetative growth and leaf area [Bertamini and Nandunchezhian 2005]. Chapman and Pratt [1961] stated that Fe level in grapevine leaves is not a reliable sign of Fe status, the best sign of Fe deficiency is symptoms in young leaves.

Total leaf blade chlorophyll content and chlorophyll a/b ratios of on 5BB and 5C grafted vines during blooming, veraison and harvest periods are given in Figures 2 and 3. Significant differences were determined between rootstocks and the phenological periods between in terms of chlorophyll content and chlorophyll a/b ratio (P < 0.01). The highest leaf chlorophyll content was measured during flowering period on both rootstocks. However, the leaf chlorophyll content of 5BB grafted vines was determined higher than on 5C grafted vines for three phenological periods. Chlorophyll contents were determined as

22.5  $\mu$ g ml<sup>-1</sup> on 5BB and 17.5  $\mu$ g ml<sup>-1</sup> on 5C grafted vines during the flowering period (fig. 2).

The leaf chlorophyll content decreased significantly increasing with leaf age. During harvest period chlorophyll content has decreased on both rootstocks. During harvest period chlorophyll content of the 5BB grafted vines was calculated as 12.9 µg ml<sup>-1</sup> and 10.9 µg ml<sup>-1</sup> on 5C grafted vines (fig. 2). According to the results of the analysis in the study, the chlorophyll a/b ratio of leaves which is grown in both rootstocks were found higher in the flowering period than veraison but lower compared to the harvest period (fig. 3). The changes in the chlorophyll a/b ratio were directly proportional to the leaf N. Indeed, many researchers report that most of the N received between flowering and veraison period [Mullins et al. 1992, Bates et al. 2002]. Conradie [1980] carried out a pot experiment with 'Chenin Blanc' cultivar and reported that post-harvest N uptake constituted about 27-37% of plant total N uptake and 60% of such amount was stored for the next season. According to our research findings while the N content of leaves declined from pre blooming to veraison, it was increased between veraison and harvest period.

# CONCLUSIONS

Grapevines were found insufficient in terms of P, K and Mg whereas they were found adequate in terms of other nutrients. The grapevines grafted on 5C rootstock were found to be favorable with regard to N, P, Ca, S, Mn and Zn contents. It is considered that in soil application of N, P, K as well as micronutrient fertilizer before bud burst and early stage of blooming will be best beneficial. Relatively higher leaf chlorophyll contents were observed in grapevines grafted on 5BB rootstock. Nutritional status of the grapevines was significantly different based on rootstocks and phenological periods. Proper fertilization should be applied before blooming to support the storage reserves of grapevines for the blooming and fruit set. In the study 5C rootstock was found to be favorable with regard to nutrient uptakes than 5BB rootstock for 'Trakya İlkeren' grape variety.

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