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RESPONSE OF GRAPEVINE (*Vitis vinifera* L.) LEAVES TO DIFFERENT LEAF FERTILIZERS UNDER A SEMI-ARID CONDITION

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Abstract. Ever-increasing shortage in global agricultural water sources urged the researchers to investigate sustainable strategies for alleviating the negative effects of drought on plants in semi arid or arid regions. In this sense, foliar fertilization gained particular significance as it supports the plant to cope with water shortage. This study was conducted to investigate the leaf physiological response of the grapevine 'Narince' to various organic leaf fertilizers. Leaf protein content was also determined to compare treatment effects on nutritional value of grape leaves since the leaves of this cultivar are consumed in various ways. Both stomatal conductance, leaf chlorophyll and protein content increased in response to leaf fertilizers in varying degrees according to the products used. To illustrate, Herbagreen pulverization resulted in the highest stomatal conductance (gs) (324.7 mmol H_2O m^{-2} s^{-1}), and was followed by Cropset (323.5 mmol H_2O m^{-2} s^{-1}) while the lowest gs value was obtained from control vines (295.4 mmol H_2O m^{-2} s^{-1}). The highest chlorophyll and protein contents were obtained from ISR 2000 (32.9 mg kg^{-1}) and Maxicrop (21.5%) treatments, respectively. Leaf chlorophyll content had a significant positive correlation with leaf protein content when all the observations were pooled. Therefore, the organic leaf fertilizers can be considered as a safe, sustainable and innovative strategies to support plants to cope with drought.

Key words: water shortage, stomatal conductance, chlorophyll concentration, leaf temperature

INTRODUCTION

Agriculture in Konya Closed Basin, coincided on the high plains of Central Anatolia, is largely dependent upon irrigation since it has a typical terrestrial climatic condition [TSMS 2016], known as hot, semiarid summers with cold and snowy winters. Increased

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production demands are rapidly depleting available surface and ground water sources. The intensive agriculture techniques based on an overuse of inputs (irrigation, fertilizer, chemicals) and improper mechanization techniques have resulted in further degradation of agriculture area. Such degradation has also decreased the organic content of the soil and increased its susceptibility to wind erosion. Wind erosion is one of the major problems in this area especially affecting the sediments remaining from an ancient shallow lake. Fertile soil is threatened to be lost completely and wind erosion also causes further humidity loss from the topsoil. Water shortage and the increasing consumption of water sources for agriculture urged the researchers to investigate sustainable strategies in semi arid or arid regions. Under drought conditions, foliar fertilization gained particular significance as it supports the plant to cope with water shortage [Hussein et al. 2013].

Studies on several plant species such as apple, apricot, grape, olive and peach, cultivated under arid environmental conditions revealed that these plant species represent different physiological strategies in stomatal response to environmental variables [Larsen et al. 1989, Johnson et al. 2009, Sabir and Yazar 2015, Sabir 2016]. Previously, the importance of investigations on the peach palm physiology has been emphasized [Clement et al. 1988], and Mora-Urpí et al. [1997] had revealed important issues for future investigations on peach palms, including the relationship among environmental and physiological variables, growth, and yield. Nonetheless, there is still insufficient knowledge about the stomatal regulation and leaf temperature of grapevines in response to leaf fertilizers. Majority of studies on the investigation of leaf stomatal behaviors have been conducted on the comparison of drought-stressed or non-stressed plants [Paranychianakis et al. 2004, Zufferey et al. 2011], while interestingly a few studies have measured the diurnal dynamics [Düring and Loveys 1996] and/or environment-dependent response of stomata [Zweifel et al. 2007].

Grape leaves, with their multifarious fillings, are a popular and widely used ingredient for dishes in southeast European and oriental kitchens. Grape leaf contains an abundant amount of vitamins, minerals and protein [Sat et al. 2002, Murtezaoglu 2006]. It has been reported that grape has important role in controlling of some liver diseases, high blood pressure and anemia. Also fibers and organic acids in grape have vital role in cleaning blood functions of digestive system and kidney [Celik et al. 1998]. The leaf has high levels of total phenols, flavonoids, flavonols, and stilbenes, all of which are known for strong antioxidant properties. Therefore, grape leaf is used to make stuffed leaves (Sarma) and forcemeat (vegetable stuffed with forcemeat) which are very popular meals in middle east countries.

The aim of this study was to investigate the leaf physiological response of the grapevine 'Narince' to various leaf fertilizers. Moreover, leaf protein content was determined to compare treatment effects on nutritional value of grape leaves.

MATERIALS AND METHODS

The present study was carried out in Implementation and Experimental Vineyard of Agriculture Faculty of Selcuk University in 2015. 'Narince', a popular Turkish white wine grape cultivar (*Vitis vinifera* L.) was used in the study. Organic fertilizer were selected on the basis of their various positive effects on plants to compare for their influences of physiology and protein content of grape leaves. According to the instructions of companies, their features and predicted effects are presented in Table 1.

Table 1. Features of organic leaf fertilizers used in the study

Materials	Features	Expected effects*
Herbagreen	micronized calcite containing $CaCO_3$(40%), SiO_2(4%), MgO (1%), and Fe_2O_3(1%)	improvement in leaf greenness, supporting the plants against abiotic stress
Cropset	*Lactobacillus acidiophilus*, plant extract, minerals	alleviating the effects of stress to stimulate healthy plant growth
ISR 2000	*Lactobacillus acidophilus*, natural bioplasm suspension of Chlorella alg cells, Bionem *Pseudomonas fluorescens*	induced resistance to biotic and abiotic stress
Maxicrop	*Ascophyllum nodosum* Seaweed based fertilizer	increase in plant vigor and foliar growth

* – according to the company instructions on produces

Study design. The long-term meteorological investigations reveal that the predominating climatic condition around the study vineyard is semi-arid with cold winters, hot and dry summers. July is the hottest month while January is the coldest one. Most of the precipitation occurs during winter and spring season. The basin usually has a relative humidity below 50%, probably because the prevailing north wind and common south wind are dry. Mean precipitation values of the years between 1950 and 2015, total annual precipitation is 320 mm. The experimental vineyard is located at 38°01.785 N, 032°30.546 E and 1158 m altitude (Central Anatolia, Turkey). The ten-year-old vines in east-west oriented rows (2 × 3 m) were trained to a typical bilateral, thin vertical shoot positioned canopy. Vines were cane pruned by leaving four or five spur canes (2 buds) per each of two arms for single vine. To investigate the effect of foliar spraying of different fertilizers on certain leaf attributes of the grapevine, an experiment in factorial format based on randomized complete block design with three replications was conducted.

Treatments were, (i) control (no foliar application), (ii) Herbagreen pulverization, (iii) Cropset pulverization, (iv) ISR 2000 pulverization, and (v) Maxicrop pulverization. The application concentration of each product was prepared according to the company instructions. Each of the treatments was performed four times during the vegetation period. The first applications for each experimental group were performed when the shoots were about 20 cm. The others were carried out with 15 days intervals. The final (fourth) application was done when the berries were approximately 0.5 mm in diameter. Cultivation practices were performed as commonly practiced by the local growers. Shoot positioning was vertical shoot positioned trellis system. The experimental vines received the same cultural practices such as weed control, and pruning and the vineyard was rain-fed with a limited precipitation around 50 ±5 kg m^{-2} occurred up to the bloom [TSMS 2016]. The pH value of the experimental vineyard was 7.5 (alkaline).

Measurements. Leaf measurements were performed 4–5 days after each pulverization. The stomatal conductance (gs), chlorophyll concentration and leaf temperatures (T_{leaf}) measurements were made on twelve leaves (6th leaf of each main shoot) from twelve individual vines around 11:00 a.m. Fully expanded but not senescent sunlit leaves at the outer canopy were chosen for measurement [Johnson et al. 2009]. As previously described by Düring and Loveys [1996] and Stavrinides et al. [2010], gs was measured near the central vein of the leaf blade with a steady state porometer (SC-1 Leaf Porometer) [Zufferey et al. 2011] and was expressed as mmol H_2O m^{-2} s^{-1}. The same area of the leaves were measured [Miranda et al. 2013], because instantaneous gs can be non-uniform over such a large leaf. T_{leaf} was also measured simultaneously with gs. Chlorophyll contents of leaves (the 3rd and 4th leaf at the shoot tips) were calculated by using the portable chlorophyll meter (Minolta SPAD-502, Japan). Forprotein analysis, twelve healthy mature leaves from the same node as the cluster per treatment were sampled at véraison stage (beginning of berry softening). The leaf samples were washed in deionized water prior to analysis. Protein content was determined based on Kjeldahl method [AOAC 2005]. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) was used to convert the measured nitrogen content to protein content.

Statistical analysis. Mean values of four time-measurements for each parameter were used for statistical analyses. The collected data were subjected to statistical analysis using a randomized factorial design. Each treatment was designed with three replicates consisting of twelve healthy vines. The mean values were compared using the least significant difference (LSD) test. Statistical tests were performed at $P < 0.05$ using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Regression analysis was performed using Pearson correlation coefficient to evaluate the correlations between certain features.

RESULTS AND DISCUSSION

Stomatal conductance (gs) responses of the grapevines to leaf fertilizers were statistically significantly different (fig. 1). Herbagreen pulverization resulted in the highest gs (324.7 mmol H_2O m^{-2} s^{-1}), and was followed by Cropset (323.5 mmol H_2O m^{-2} s^{-1}). On the other hand, the lowest gs value was obtained from untreated control vines (295.4 mmol H_2O m^{-2} s^{-1}), indicating the positive effects of leaf pulverizations on stomatal gas exchange of the leaf. The gs refers to the amount of CO_2 passing in and water vapor passing out of leaves of grapevines and so, has consequences on photosynthetic rate and transpiration. In a study conducted under glasshouse conditions in Turkey, the gs values for the cultivars 'Italia', 'Crimson Seedless' and 'Alphonse Lavallée' grapevine cultivars were 287.7, 262.1 and 242.0 mmol H_2O m^{-2} s^{-1}, respectively. Similar, Zsófi et al. [2014] found the gs range between 197 and 269 mmol H_2O $m^{-2}s^{-1}$ for Hungarian grapevine cultivar. Considering the mentioned studies, the gs values of the 'Narince' vines in the present study are quite higher than those obtained from different cultivars. Such differences may be due to ecological and genotypic differences. In addition, leaf pulverizations led to increases the gs of the vines in varying extent depending on the products used.

As illustrated in Figure 2, the leaf chlorophyll content of 'Narince' wine grape cultivar was significantly affected by different leaf fertilizers. All the leaf treatments increased the chlorophyll content of the leaves. The highest chlorophyll content was obtained from ISR 2000 (32.9 mg kg^{-1}) and was followed by Herbagreen (32.4 mg kg^{-1}), while the lowest value was obtained from control vines (30.5 mg kg^{-1}). Similarly, Kara and Sabir [2010] determined obvious vitalization of the green mass of the leaves with dark green color. The findings would imply that Herbagreen might accelerate the photosynthetic activity in leaves.

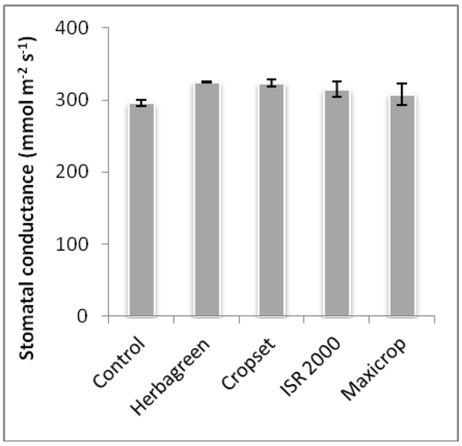

Fig. 1. Leaf stomatal conductance (gs) responses of the grapevines to leaf fertilizers

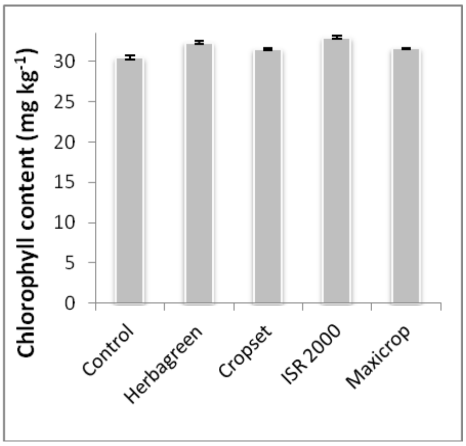

Fig. 2. Leaf chlorophyll content responses of the grapevines to leaf fertilizers

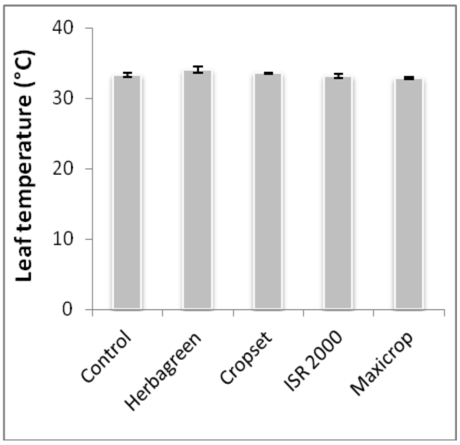

Fig. 3. Leaf temperature (T_{leaf}) responses of the grapevines to leaf fertilizers

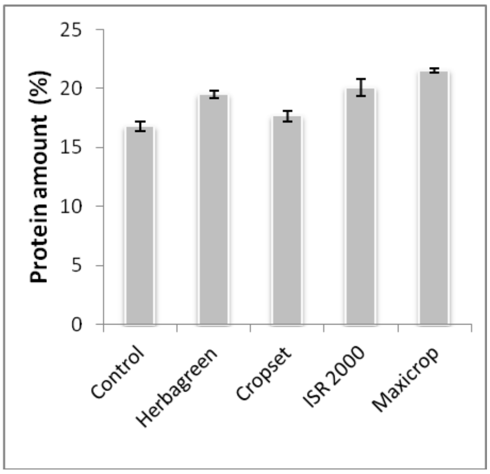

Fig. 4. Leaf protein amount responses of the grapevines to leaf fertilizers

The leaf temperature response of 'Narince' grape to different leaf fertilizers is depicted in Figure 3. Although the differences were statistically insignificant, the leaf temperature values of the vines of ISR 2000 and Maxicrop treatments were sightly lower than those of control vines, while the vines treated with Herbagreen or Cropset had higher values. Overall leaf temperature results indicate that all the values on leaf temperature are slightly higher than certain literature report [Rogiers et al. 2011, Greer 2012, Sabir and Yazar 2015], possibly indicating that the experimental vines were in slight drought stress in accordance with the results of Hirayama et al. [2006]. In many cases, high leaf temperature is an indication rate which can display plant water stress.

Plants with high water use efficiency are able to thrive in water limiting soils and consequently keep their leaf temperature moderate [Liu and Stutzer 2002, Gonzalez-Dugo et al. 2005]. The vineyard of present experiment is located in Konya basin under a semi-arid climatic condition with a total annual precipitation of 378 mm in 2015 [TSMS 2016]. Considering this value, the irrigation demand far exceeds the potential water capacity. On the other hand, the highest daily air temperature was determined on 31 July (35.3°C) [TSMS 2016].

Table 2. Regression table with significance levels

	Protein amount	Leaf temperature	Stomatal conductance
Chlorophyll content	0.589*	0.127	0.508*
Stomatal conductance	0.159	0.338	
Leaf temperature	-0.276		

Significant values are indicated with * for $P < 0.05$ level

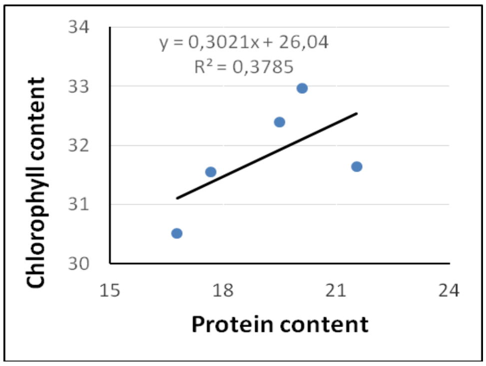

Fig. 5. Correlation between leaf chlorophyll content and stomatal conductance

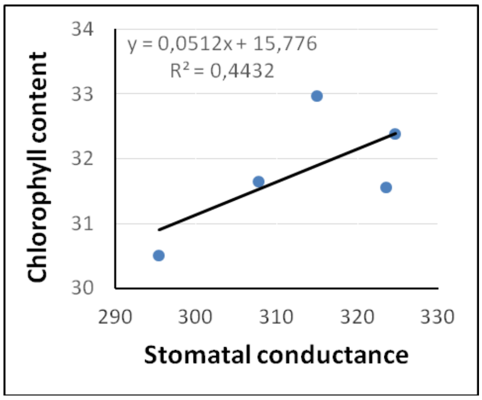

Fig. 6. Correlation between leaf chlorophyll content and leaf protein amount

All the leaf pulverization treatments improved the leaf protein content in varying levels (fig. 4). Differences among the treatments were statistically significant. Spraying the grapevine leaves by Maxicrop resulted in the highest protein amount in leaves (21.5%), and was followed by ISR 2000 treatment (20.1%), while the lowest protein level was determined in untreated control vines (16.8%). Studying on protein content of various grape leaves, Murtezaoglu [2006] determined a wide range from 11.6 to 24.79%, depending on the cultivars. She concluded that the grapevine leaves possess a high nutritional value with their high protein content. The correlation between leaf chlorophyll content with other leaf physiological parameters such as stomata conductance and leaf temperature has recently used to understand the plant physiology and stress level [Kapotis et al. 2003, Papasavvas et al., 2008]. Regression analysis (r, the Pearson correlation coefficient) results (tab. 2) revealed that leaf chlorophyll content had significant positive correlations with leaf stomatal conductance (fig. 5) and protein content (fig. 6). Correlation data proven that both leaf chlorophyll and protein content increased similarly in response to leaf fertilizers. A similar positive correlation between chlorophyll concentration and protein content in potato leaves was also detected by Botha et al. [2006] who studied on non-destructive estimation of leaf chlorophyll and protein contents. Matsumoto et al. [2005] indicated that the stomatal conductance variability depended markedly on chlorophyll function in *Quercus serrata* trees. They further emphasized that a reduction in the chlorophyll concentration induced a hyperbolic decrease in stomatal conductance, while a low leaf chlorophyll concentration imposed a restriction on the opening capacity of the stomata. Papasavvaset al. [2008] found a linear correlation between chlorophyll content and the physiological parameters of the leaves, such as photosynthesis, transpiration and stomata conductance of *Beta vulgaris*. It has been well established that stomata are the primary structures that exchange water and CO_2 between plants and the atmosphere. Therefore, stomatal conductance is an important factor in the cycling and balancing of water, CO_2, and energy between plants and the atmosphere. The results of the present and the mentioned studies verify the fundamental correlation between chlorophyll content and the physiology of the plant leaf.

CONLUSION

Recently, foliar fertilization gained particular significance for environment-friendly mitigation of the negative impacts of global water shortage and climate change on plants. In the present study, stomatal conductance, chlorophyll and protein contents of the leaf of 'Narince' grapevine cultivar cultivated under semi-arid climatic condition were markedly improved by leaf fertilizers containing various natural products. Considering the overall investigations, Herbagreen (micronized calcite) and ISR 2000 (bioplasm suspension of Chlorella alg cells) might especially be recommended as safe, sustainable and innovative strategies to support plants to cope with environmental stress factors.

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REAKACJA LIŚCI WINOROŚLI (*Vitis vinifera* L.) NA RÓŻNE NAWOZY DOLISTNE W WARUNKACH PÓŁPUSTYNNYCH

Streszczenie. Zwiększające się niedobory źródeł wody dla rolnictwa skłoniły badaczy do analizowania strategii zrównoważonych w celu zmniejszenia negatywnego wpływu suszy na rośliny w regionach półpustynnych lub pustynnych. W tym kontekście, nawożenie dolistne zyskało szczególne znaczenie, gdyż wspiera roślinę w radzeniu sobie z brakiem wody. W doświadczeniu badano reakcję fizjologiczną liści winorośli ‘Narince’ na różne organiczne nawozy dolistne. Określono też zawartość białka w liściach, aby porównać efekty zabiegów na wartość odżywczą liści winogron, gdyż liście tej odmiany są spożywane na różne sposoby. Przewodność szparkowa (gs) oraz zawartość chlorofilu i białka w liściach zwiększały się w reakcji na nawozy dolistne w różnym stopniu, w zależności od użytych produktów, np. sproszkowany Herbagreen dał najwyższy gs (324,7 mmol H_2O m^{-2} s^{-1}), na drugim miejscu był Cropset (323,5 mmol H_2O m^{-2} s^{-1}) natomiast najniższą wartość gs osiągnięto z kontrolnych winorośli (295,4 mmol H_2O m^{-2} s^{-1}). Największą zawartość chlorofilu i białka osiągnięto, odpowiednio, z zabiegów ISR 2000 (32,9 mg kg^{-1}) i Maxicrop (21,5%). Po zebraniu wszystkich danych okazało się, że zawartość chlorofilu w liściach była pozytywnie skorelowana z zawartością białka w liściach. Organiczne nawozy dolistne mogą więc być uważane za bezpieczne, zrównoważone i innowacyjne strategie wspierania roślin podczas suszy.

Słowa kluczowe: brak wody, przewodność szparkowa, stężenie chlorofilu, temperatura liści

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