

## PHYSIOLOGY AND VEGETATIVE DEVELOPMENT OF ‘BLACK MAGIC’ GRAPEVINES IN RESPONSE TO DIFFERENT ROOTSTOCKS

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**ABSTRACT.** The grape cultivars and rootstock show great variation in terms of physiology and vegetative development. Response of grapevines to environmental conditions has been affected by various factors such as the rootstocks used in grafting. Therefore, the aim of this study was to determine the rootstock effects on the vegetative growth and physiology of ‘Black Magic’ (BM) by comparing the grafted vines with ungrafted cultivar in calcareous soil under continental climate condition. The results showed that all the physiological and growth parameters evaluated in the study displayed great variations. Stomatal conductance and leaf temperature of the scion was obviously decreased by the rootstocks 44-53 M and R. du Lot. Shoot length was remarkably greater in BM/41 B grafting than other rootstocks or grafting combinations. Shoot length, leaf number and leaf area of the scion were higher on rootstocks (except for leaf area of BM/R. du Lot) than its own rooted vine. Rootstocks had significant promotion on the development of ‘Black Magic’ grown in calcareous soil under continental climate condition. 41 B displayed higher contributions to many growth parameters of the scion under this condition and thus appeared to be preferable one among them according to these preliminary investigations.

**Keywords:** *Vitis vinifera* L., viticulture, rootstock, grafting, scion cultivar

### INTRODUCTION

Environmental stress factors and global climate change became serious problems restricting the sustainability of agricultural production [1]. High levels of temperature increase, water shortages, unexpected heavy rains, storms, flooding and hails are some of the consequences of the global climate changes. Such events cause degradation in cultivated soil and depletion in the physiology and metabolism of the plants. Considerably significant part of existing vineyards around the world has been found on land that has continental climate conditions [2], where arid or semiarid conditions adversely affect the plant physiology and fruitfulness [3]. Furthermore, phylloxera (*Daktulosphaira vitifoliae* Fitch), a plant sap-sucking and gall-forming insect, collapsed the French viticulture sector in the late 19th century, destroying a third of all vineyards [4]. This is a destructive pest for global viticulture as an obligate biotroph of *Vitis* species, on which it can infest both roots and leaves. Phylloxera that has so viciously attacked the roots of European grapevine cultivars (*Vitis vinifera* L.) worldwide continues to munch its way from vine to vine. Grapevine rootstocks have been advised to be a prime solution to fight with gall-forming aphid phylloxera as *Vitis vinifera* L. cultivars do not have genetic resistance. A wide range of rootstocks with their phylloxera resistant root systems have being bred to cope with this pest. Today, growers have to graft *V. vinifera* L. grape cultivars onto rootstocks developed from North American species [5,6], using the genotypes mainly belonging to *V. rupestris*, *V. riparia*, *V. berlandieri* and *V. champinii* by certain breeders with particular targets. Therefore, each genotype has unique features relevant to environmental biotic and abiotic stress factors. Comprehensive investigations on the genetic resistance potential against many stress factors indicated that

rootstock genotypes possess invaluable genetic sources with differing attributes [7]. Upon increasing awareness about the use of rootstocks for challenging the adverse effects of biotic and abiotic stressors, grape growers commenced using rootstocks for sustainable grape production on the face of ever increasing climate change. In grape propagation, grafting incorporates a new grape cultivar (called the scion), onto the root system of a desired rootstock genotype for various purposes such as better adaptation to phylloxera, nematode, high pH, salination, etc. Grapevine rootstocks have a great genetic variability [7], as they have been developed from a wide range of species [5], providing the viticulturists to choose the proper one according to a given ecology. Grapevine rootstocks display a significant disparity in terms of the response to environmental factors. Grafting affinity is also an important physiological issue that affects the vigor and fruitfulness of the grafted grapevines. For a sustainable viticulture on the face of ever increasing global climate change, choice of an appropriate rootstock is a key factor for appropriate resistance to environmental stresses because of its influence on the physiology and growth properties of scion cultivar [8]. This issue becomes more important when a newly introduced grape cultivar is considered to cultivate in stressful ecology. Therefore, this study was conducted to compare the physiology and growth characteristics of grafted and ungrafted young grapevines of 'Black Magic' cultivar using 41 B, 44-53 M and Rupestris du Lot rootstocks in a one year old vineyard established on a calcareous soil under the continual climate condition.

## MATERIALS AND METHODS

### *Study Design*

A vineyard study was established in the Research and Implementation Vineyard of Selcuk University located at 38°01.785 N, 32°30.546 E and 1158 m above sea level (Central Anatolia, Türkiye). Own rooted *V. vinifera* L. cultivar 'Black Magic' grapevine plants were compared with three grafting combinations using the rootstocks; 1) 41 B (*V. vinifera* x *V. berlandieri*), 2) 44-53 M [*Vitis riparia* x 144 M (*V. cordifolia* x *V. rupestris*)], and 3) Rupestris du Lot (*V. rupestris*) in terms of the effects of rootstock uses on physiological and growth features. One year old plants of each grafting combination including own rooted 'Black Magic' plants were transplanted into vineyard with calcareous soil condition under continental climate condition. The long term climatic data collected from 1929 to 2020 by Turkish State Meteorological Service [9] indicate that climatic condition in the research vineyard is arid/semi-arid with cold winters, hot and dry summers. The highest and lowest mean temperatures are 18.0 and 5.4 °C, respectively, with an annual mean temperature of 11.7 °C. Minimum and maximum temperatures were -28.2 °C and 40.6 °C, respectively. Mean precipitation is 329.2 mm, with a relative humidity below 50%. The soil characteristic of experimental vineyard is calcareous with a high pH  $\approx 7.9 \pm 0.2$ ) and clay loamy texture. One year old grapevine plants with approximately  $30 \pm 5$  cm single summer shoots were transplanted into the vineyard at the beginning of the summer season in 2023. The grapevine rows were east–west oriented with the rectangular transplantation spacing of 1.5 and 3.0 m within grapevines and between rows, respectively. The summer shoots were vertically positioned using wires for optimum and equally benefiting from sunlight.

### *Measurement and Analyses*

Measurements on leaf stomatal conductance (gs) and leaf temperature ( $T_{\text{leaf}}$ ) were carried out periodically during the summer season using the healthy leaves born at 5<sup>th</sup> to 7<sup>th</sup> nodes of each shoot from all vines between 09:00 and 12:00 h [10]. A fully expanded sun exposed leaf per plant was used for the gs and  $T_{\text{leaf}}$  investigations [11]. The gs and  $T_{\text{leaf}}$  was measured near

the central vein on blade with a portable leaf porometer (SC-1 Leaf Porometer) [12] and was expressed as  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ .

Shoot length and shoot lignification length (the length of the scion shoot, where complete lignification occurred) was measured with a tape measure having a sensitivity of 1 mm). Shoot diameter was recorded by digital caliper at a point 1 cm above the second node. Leaf number was counted as the total leaf (or node) on the plant shoot [6].

Chlorophyll contents of the newly expanded mature leaves (third and fourth leaves at the shoot tip) were determined with a portable chlorophyll meter (SPAD-502, Minolta, Japan). Measurements on area of nine fully expanded mature leaves per plot were performed when the shoot growth was near to cease in the late summer. Using one set of nine leaves, leaf area was estimated with WinFolia computer image analysis system. Leaf fresh weights were determined with a balance with a 0.001 g precision using another set of nine mature leaves. The relevant fresh leaf samples were then heated in 105 °C at 24 h to obtain leaf dry weight. After weighing, fresh leaves were hydrated to near maximum turgor by immersing in distilled water for four hours to ensure full rehydration. At the end of rehydration period, leaf samples were weighed to obtain final turgid mass (TM) and placed in an oven [13], at 70 °C for 48 h in order to obtain DM. All mass measurements were made using an analytical scale, with precision of 0.0001 g. Values of FM, TM, and DM were used to calculate RWC, using the equation suggested by Pieczynski et al. [14]:

$$\text{RWC (\%)} = \frac{[\text{FM} - \text{DM}]}{[\text{TM} - \text{DM}]} \times 100.$$

Weight measurements were carried out with an analytical scale having a precision of 0.0001 g [15].

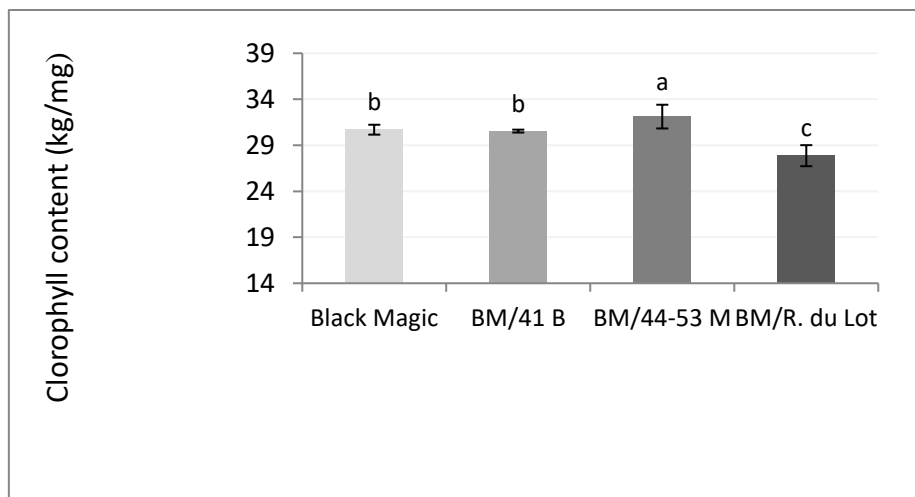
### ***Statistical Analysis***

The collected numerical data were subjected to statistical analysis using a randomized factorial design. Each experimental plant plot was established with three replicates composed of three healthy uniformly grown vines. The comparison of mean values was performed using the least significant difference (LSD) test. Statistical tests were carried out at  $P < 0.05$  using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Regression analyses were performed to reveal the relationships between certain physiological properties.

## **RESULTS AND DISCUSSION**

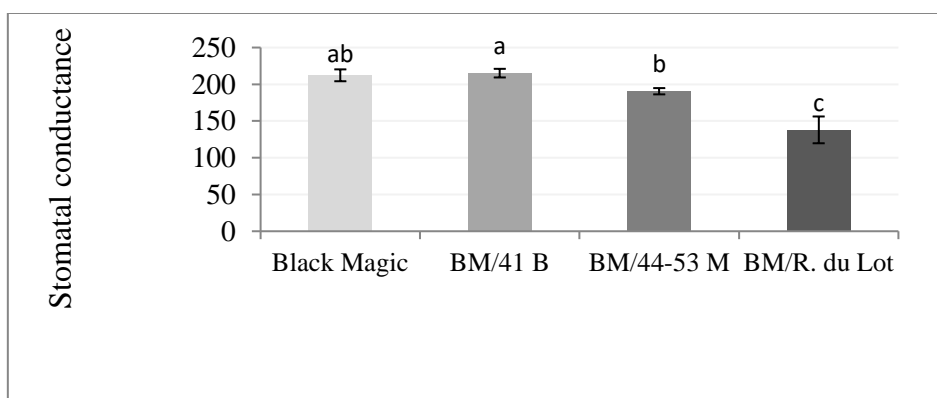
### ***Physiological Investigation***

The leaf chlorophyll content displayed significant variation among the grapevines (Fig. 1). The highest chlorophyll content was found in BM/44-53 M grafting combination (32.1 mg/kg) while the lowest chlorophyll values were determined in BM/du Lot (27.9 mg/kg). The chlorophyll content in BM/41 B was just similar to that of ungrafted BM vines, probably due to closeness in their genetic background as the maternal genotypes of 41B is *V. vinifera* cv. 'Chasselas'. The overall chlorophyll content values across the grapevines are within the general findings recorded on different grapevine rootstocks [15,16] and cultivars [17]. This indicates that the grapevines synthesized satisfactory chlorophyll pigments in the leaves under this stressful ecological condition.



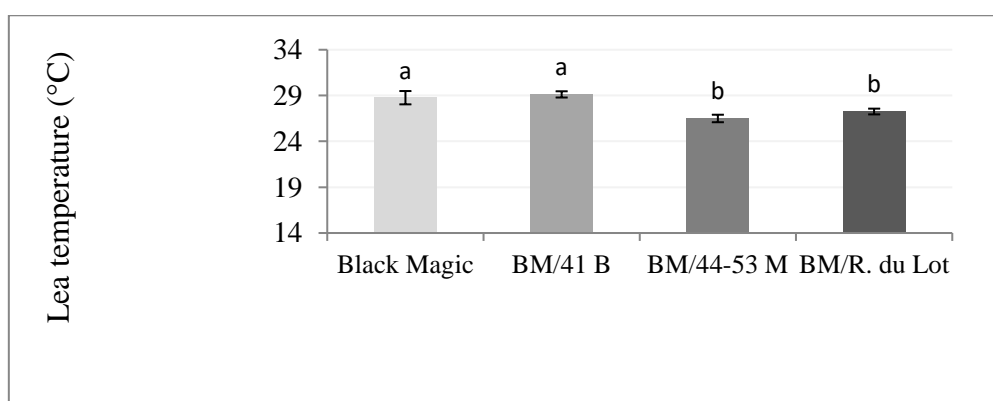
**Fig. 1.** Changes in leaf chlorophyll content (mg/kg) of the grapevines as influenced by different rootstocks. Each column represents the mean of triplicate observations ( $n = 9$ ). Error bar represents the standard deviation of that mean ( $P < 0.05$ ,  $LSD = 1.02$ ).

Stomatal conductance (gs) displayed significant variation among the grapevines (Fig. 2). The highest gs was determined in BM/41 B ( $215.1 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ), while the lowest gs was found in BM/R. du Lot ( $137.9 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ) followed by BM/44-53 M ( $190.5 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ). Similar to the chlorophyll content, the gs of BM/41 B was just similar to that of ungrafted BM vines. Stomata have significant role for regulating the exchange of water and energy between plants and the atmosphere. In the context of climate warming, particularly in arid and semiarid regions, the accurate knowledge of the gs variation patterns is essential to the study of crop evapotranspiration, productivity and drought resistance characteristics [18]. As known, grapevine rootstocks can modify the leaf gas-exchange of the scion [19] through modulating elemental acquisition, metabolomics profile, and the shape of canopy in the scion [20] although the experimental knowledge on magnitude and change trends of such effects was unclear in the literature. The present study revealed that grapevine rootstocks 44-53 M and R. du Lot tended to decrease the gs of scion ‘Black Magic’. Therefore, these findings along with the aforementioned literature indicate that grapevine rootstocks have significant effects on stomatal regulation and drought resistance of the scion cultivar.



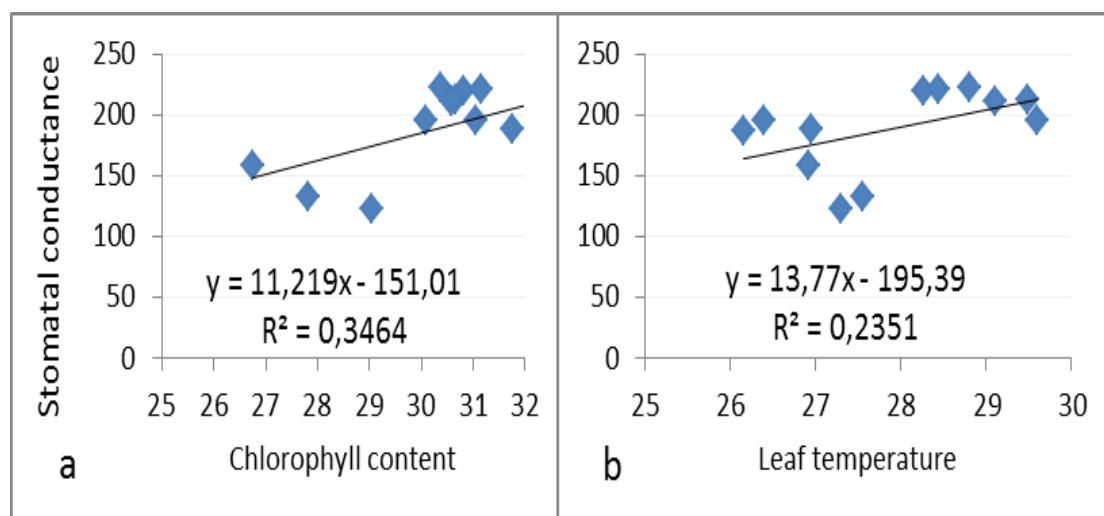
**Fig. 2.** Changes in leaf stomatal conductance ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ ) of the grapevines as influenced by different rootstocks. Each column represents the mean of triplicate observations ( $n = 9$ ). Error bar represents the standard deviation of that mean ( $P < 0.05$ ,  $LSD = 22.7$ ).

Leaf temperature ( $T_{leaf}$ ), one of the prime factors affecting the plant physiology [10] and reflecting the stress level of plants [21], showed significant variation among the grapevines (Fig. 3). The highest  $T_{leaf}$  values were found in BM/41 B and BM grapevines with very close values (29.1 and 28.8 °C, respectively). The lowest  $T_{leaf}$  was determined in BM/44-53 M (26.5 °C) followed by BM/R. du Lot (27.3 °C). The overall values indicated that the rootstocks had direct influences on  $T_{leaf}$  of BM cultivar. Marguerti et al. [21] reported that scion leaf temperature, transpiration rate and its acclimation to water shortage are controlled genetically by the rootstock. Thus, variation in  $T_{leaf}$  of BM grapevines on different root systems may possible be due to different effects of rootstocks on transpiration rates and cooling of the scion. The range of  $T_{leaf}$  was within the threshold values for optimum photosynthesis in grapevines (25–30°C) suggested by Greer [22].



**Fig. 3.** Changes in leaf temperature (°C) of the grapevines as influenced by different rootstocks. Each column represents the mean of triplicate observations ( $n = 9$ ). Error bar represents the standard deviation of that mean ( $P < 0.05$ ,  $LSD = 0.89$ ).

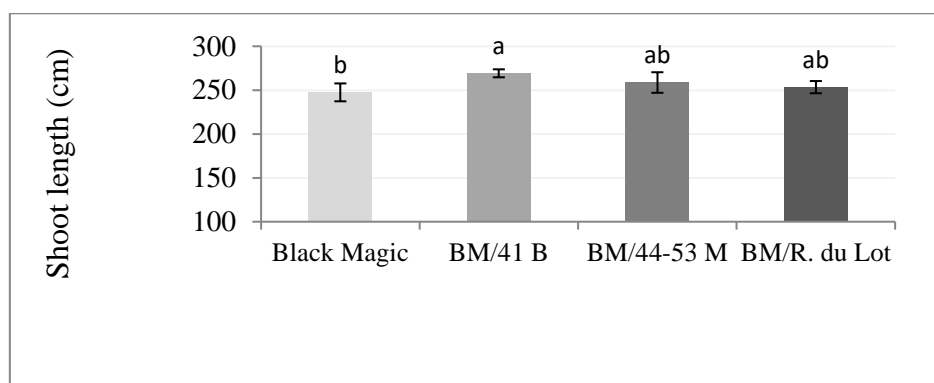
Chlorophyll content,  $g_s$  and  $T_{leaf}$  are the key physiological features affecting the net photosynthetic rate in plant leaf. According to the regression analysis shown in Fig. 4a and b,  $g_s$  showed significant linear relation with leaf chlorophyll content as previously reported by Sabir et al. [16] and Dilek and Sabir [15], using different grapevine genotypes. There was a weak linear correlation between  $g_s$  and  $T_{leaf}$ . Therefore, stomatal conductance variability depended markedly on chlorophyll function.



**Fig. 4.** Regression analyses between stomatal conductance ( $g_s$ ) and chlorophyll content (a) and  $g_s$  and leaf temperature ( $T_{leaf}$ ) (b).

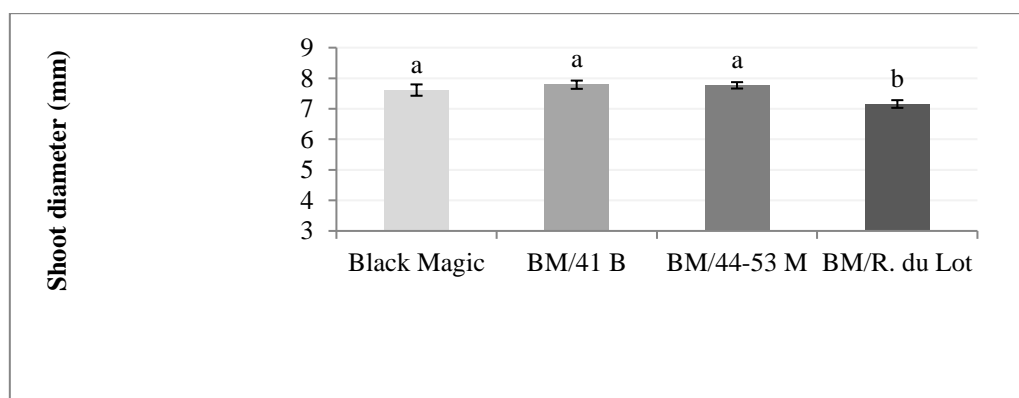
## Growth Investigations

Shoot length significantly differed among the grapevines (Fig. 5). The highest shoot length was obtained from B. Magic/41 B (269.1 cm), while the lowest shoot length values were found in own rooted B. Magic (247.4 cm) and BM/R. du Lot (253.3 cm). The length of the young summer shoots is one of prime parameters that determine the initial vegetative vigor and subsequent resistance of newly transplanted grapevines to harsh conditions such as winter cold and summer drought. The shoot length findings across the studied vines are within the general values found in literature [15,23] and, therefore, their first year developments seem logical or young vines. Under continental climate conditions, like Konya, a well-balanced vegetative development with lignified shoot growth is a key factor to resist the winter cold that can be as low as  $-20^{\circ}\text{C}$  for most winter days [9].



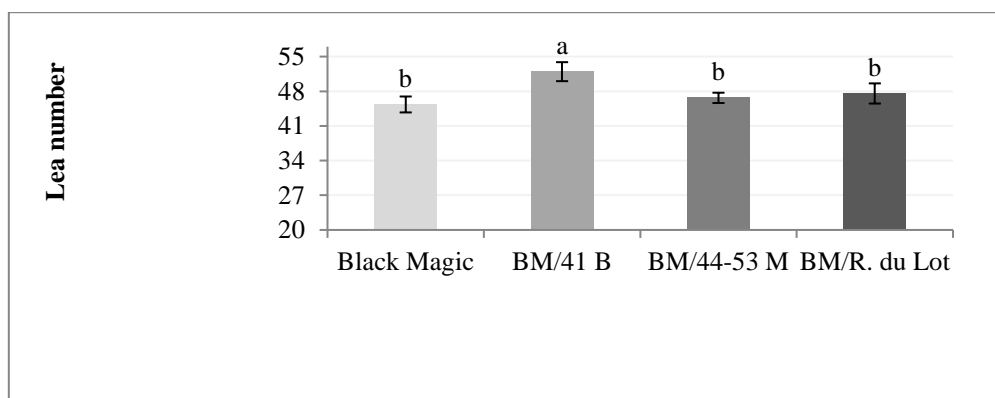
**Fig. 5.** Changes in shoot length (cm) of the grapevines as influenced by different rootstocks. Each column represents the mean of triplicate observations ( $n = 9$ ). Error bar represents the standard deviation of that mean ( $P < 0.05$ ,  $LSD = 16.62$ ).

As illustrated in Fig.6, shoot diameter significantly varied in response to the rootstock use. The highest shoot diameter was found in BM/41 B which was followed by BM/44-53 M with very close values. The lowest shoot diameter was measured in BM/R. du Lot. Shoot diameter values were similar to those of Zengin and Sabır [15] who studied on various *Vitis* genotypes. Shoot diameter is reported to be effective on cold hardiness in grapevines [24]. Therefore, grape growers and breeders may consider the importance of the shoot diameter for grape production or breeding studies in cold climates.



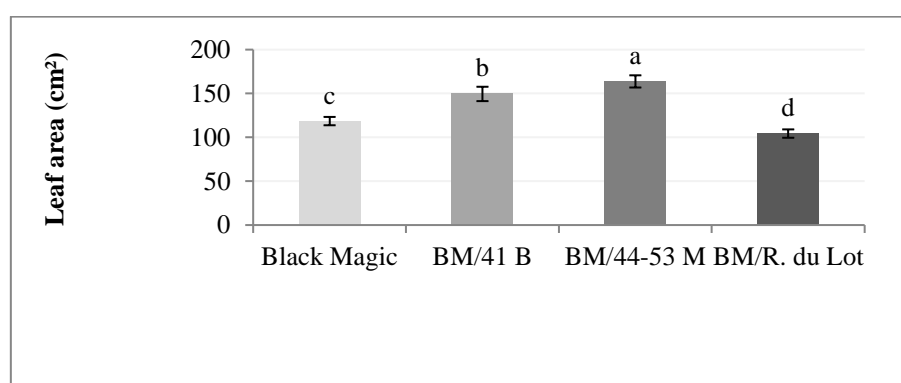
**Fig. 6.** Changes in shoot diameter (mm) of the grapevines as influenced by different rootstocks. Each column represents the mean of triplicate observations ( $n = 9$ ). Error bar represents the standard deviation of that mean ( $P < 0.05$ ,  $LSD = 0.27$ ).

The rootstock use resulted in significant variation in leaf number per shoot (Fig. 7). The highest leaf number on summer shoot was counted in BM/41 B (51.9 leaves) while the lowest leaf number was counted in own rooted vines (45.3 leaves). As expected, leaf number findings just resembled to those of shoot length data for the rootstock dependent increase and decrease trends. In comparison to the ungrafted 'Black Magic' plants, a 12.7% increases in leaf number of BM/41 B vines was obtained.



**Fig. 7.** Changes in leaf number of the grapevines as influenced by different rootstocks. Each column represents the mean of triplicate observations ( $n = 9$ ). Error bar represents the standard deviation of that mean ( $P < 0.05$ ,  $LSD = 3.18$ ).

As depicted in Fig. 8, leaf area displayed a significant and wide range of variation as the grapevine genotypes have been originated from various genetic backgrounds. The greatest leaf area was obtained from BM/44-53 M grapevines ( $163.8 \text{ cm}^2$ ), followed by BM/41 B ( $149.5 \text{ cm}^2$ ). Grapevine development can be characterized in several different ways one of which is leaf growth that directly influence the photosynthetic activity of plants [25]. However, disparities between shoot length and leaf area findings implied that the leaf growth alone is not as sufficient as leaf physiology for assessment of vegetative development.



**Fig. 8.** Changes in leaf area ( $\text{cm}^2$ ) of the grapevines as influenced by different rootstocks. Each column represents the mean of triplicate observations ( $n = 9$ ). Error bar represents the standard deviation of that mean ( $P < 0.05$ ,  $LSD = 11.9$ ).

As presented in Table 1, leaf fresh weight, leaf dry weight and leaf relative water content (RWC) of the grapevines were significantly influenced by the rootstock. The greatest values in these parameters were obtained from the vines of BM/41 B grafting. Ungrafted plants and BM/R. du Lot grapevines showed similar values for these features.

**Table 1.** Changes in leaf fresh weight (g), leaf dry weight (g) and leaf relative water content (%) of the grapevines as influenced by different rootstocks.

	Fresh weight (g)	Dry weight (g)	Relative water content (%)
'Black Magic'	2.95±0.46 c	0.82±0.14 c	88.6±1.06 b
BM/41 B	5.42±0.69 a	1.55±0.12 a	91.6±1.41 a
BM /44-53 M	4.01±0.40 b	1.03±0.14 b	88.5±1.28 b
BM/R. du Lot	2.89±0.07 c	0.68±0.05 c	86.2±1.91 c
LSD (%5)	0.23	0.17	1.74

## CONCLUSION

In this study, plant physiology and vegetative development of grapevines were investigated with comparing the grafted and ungrafted young grapevines of 'Black Magic' cultivar in a suboptimal soil (pH ≈ 8.02) under the continental climate condition. Expectedly, own rooted grapevines of 'Black Magic' cultivar and their grafting combinations displayed significant differences in terms of plant physiology and vegetative development due to distinctness in their genetic origin. Among the rootstocks, 41 B was the most effective one on promoting shoot development of the scion cultivar. Therefore, this study implies that 41 B may be preferred for sustainable viticulture under the similar conditions of this study. Nonetheless, long term investigations would yield further information about the behaviors of different rootstock genotypes under various stress factors.

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