Tarım Bilimleri Araştırma Dergisi 7 (1): 39-44, 2014
ISSN: 1308-3945, E-ISSN: 1308-027X, www.nobel.gen.tr

The Roles of Arbuscular Mycorrhizal Fungi on Some Growth Parameters and Biochemical Compounds on Some Vitis Rootstock

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Received: April 03, 2014
Accepted: May 10, 2014

Abstract
Viticulture constitutes a highly important branch of agriculture production in the world. As it is known viticulture, which can be carried out in a fairly large area, was seriously damaged after the appearance of the phylloxera. Later on, with the determination that American grapevine rootstocks are resistant against this pest, vineyards started to be established with species grafted on these rootstocks and many rootstocks were bred. However, the rootstocks used today have also some disadvantages as well as the advantages they bring in. The primaries of these disadvantages are the poor rooting characteristics and low tolerance shown to some soil conditions. In the present study it was aimed to determine the effects of arbuscular mycorrhizal fungi (AMF) on rooting characteristics and some biochemical compounds of rootstocks. Varying concentrations (10-20 g/l) of AMF were applied to cuttings of Kober 5 BB, 41 B and 110 R American rootstock and the effects of the application on some growth parameters (rooting percentage, shoot length and shoot weight) and some biochemical compounds (chlorophyll, soluble sugar and phenolic compound) were determined. As a conclusion, it was determined that AMF promote shoot and root development of American grapevine rootstocks and that they have a significant potential in terms of biochemical compounds making them potent protector from some stress conditions.
Key words: Mycorrhiza, grapevine rootstock, chlorophyll, soluble sugar, phenolic compound

Bazı Amerikan Asma Anaçlarında Arbuskular Mikorizal Fungusların Bazı Gelişim Parametreleri ve Biyokimyasal Bileşikler Üzerine Rolleri

Özet
Bağcılık dünyada oldukça önemli tarımsal üretim kollarından birisini oluşturmaktadır. Geniş alanlara yayılmış olan bağcılık bilindiği gibi filoksera zararlısının görünmesinden sonra ciddi bir zarara uğramıştır. Amerikan asma anaçlarının bu zararlıya dayanıklı olduklarının belirlenmesi üzerine de bağlar bu anaçlar üzerine aşılı çeşitlerle kurulmaya başlanmış ve bu amaçla çok sayıda anaç ıslah edilmiştir. Bununla birlikte günümüzde kullanılan anaçların avantajlarına karşın bir takım dezavantajları da bulunmaktadır. Bu dezavantajların başında köklenme yeteneklerinin düşük olması ve bazı toprak koşullarına toleranslarının düşük olması gelmektedir. Bu çalışma da arbuskular mikorizal fungusların (AMF) anaçların köklenme yetenekleri ve bazı biyokimyasal bileşikler üzerine olan etkilerinin belirlenmesi amacıyla gerçekleştirilmiştir. Kober 5 BB, 41 B ve 110 R Amerikan asma anacına ait çeliklere iki farklı konsantrasyonda (10-20 g/l) AMF uygulanmış ve bazı gelişme parametreleri (köklenme yüzdesi, sürgün gelişimi ve sürgün uzunluğu) ile bazı biyokimyasal bileşikler (klorofil, çözünebilir şeker ve fenolik bileşik) üzerine olan etkileri belirlenmiştir. Araştırmada sonuç olarak AMF nin Amerikan asma anaçlarında sürgün ve kök gelişimini teşvik ettiği ve özellikle bazı stres koşullarından korunmada etkili olan biyokimyasal bileşikler bakımından da bir potansiyel olduğu belirlenmiştir.
Anahtar kelimeler: Mikoriza, asma anacı, klorofil, çözünebilir şeker, fenolik bileşik

INTRODUCTION

Viticulture that reaches back 5000 to 6000 years before Common Era has a highly important position in the history of agriculture. Grapevine can be cultivated in widely different geographical areas and it is believed that the number of varieties cultured in the world is about 14.000 [1]. Before the phylloxera pest contaminated European vineyards from America in 1863, propagation of grapevine had been carried out by rooting cuttings of *Vitis vinifera* species. However, with the emergence of phylloxera this method, which now is known as "the old viticulture", was abandoned and it became necessary to establish new vineyards by grafting local species on American grapevine rootstocks that are determined to be resistant against this pest. Even today, this method that is referred to as the "modern viticulture" is used as the only method for grapevine propagation. Positive effects of the used rootstocks on the growth, development, yield and quality of the species and the provision of resistance against various stress factors are of great importance. Many rootstocks are bred and it is known that these rootstocks exhibit varying levels of resistance against various stress factors including drought, lime, nematode, salinity and particularly phylloxera. However, it is naturally not possible to have all of the desired qualities at the same time in the same rootstock. Due to this reason selecting the rootstock that will adapt in the best way possible to the current climate and soil conditions and the species grafted on it, and in addition carrying out applications that minimize the negative effects of stress factors are of great importance. One of the applications that have positive effects on plant growth and development, and that have potential in terms of preventing or mitigating stress is to

ensure Arbuscular Mycorrhizal Fungi (AMF) - plant interactions.

It is known that, living a symbiotic life where mutual benefits with plants are in question, AMF receive carbon from host plant as photosynthesis product and in return contribute the nourishment of the plant by enabling the absorption of phosphorus and other nutritional elements from the soil. In consequence, the root-shoot development as plant growth parameters are naturally affected and increase in leaf chlorophyll is achieved [2]. It is also known that AMF enhance host plants' tolerance against biotic and abiotic stress conditions such as pathogens [3], heavy metals [4,5,6] or drought [7].

It is known that some changes in the soluble sugar [8,9,10,11] and phenolic compound contents of plants may take place under stress conditions and the damages caused by stress on plants are reduced at the extend of these changes. It is believed that AMF have a significant role in the synthesis of these compounds. As a matter of fact, it is known that as a reaction to the penetration of the root by AMF, compounds such as arginine and isoflavonoids [12] and soluble sugar content [11] are increased in the root.

As mentioned above, AMF have multiple functions on the constitution of plants. However, there are only a limited number of studies conducted for determining their effects on American grapevine rootstocks. Yet, it is essential to determine the effects of AMF on plant development parameters and accordingly on stress parameters, and to put the gained information into practice. For this purpose, varying concentrations (10-20 g/l) of AMF were applied on Kober 5 BB, 41 B and 110 R American grapevine rootstock cuttings, and the effects of the application on certain plant growth parameters such as rooting percentage, shoot length and weight, and chlorophyll, soluble sugar and total phenolic compound contents were determined in the present study.

MATERIAL AND METHODS

In this study, cuttings belonging to Kober 5 BB (Berlandieri x Riparia Teleki 8 B), 41 B (Chasselas x Berlandieri 41 B) and 110 R (Berliandieri Ressêguier No. 2 x Rupestris Martin 110 Richter) American grape rootstocks were used as plant material. The materials have been supplied from Egirdir Fruit Research Station (Isparta/Turkey). The length of the cuttings is 35-45 cm, their thickness is 7-10 mm, they have been prepared so that they can have 3-5 buds on themselves, the other buds except the uppest bud have been dulled. The cuttings have been planted to polyethylene sacks (12 x25 cm), they that include perlite:turf mixture in the ratio of 1:1 and they have been cultivated in the incubation room in 24±1°C temperature, 16/8 hours brightness/darkness periods. Coincidence parcels have been arranged depending on the test pattern with three replication and 15 cuttings have been used in each replicate. One month after the planting, the cocktail (*Glomus intraradices, Glomus aggregatum, Glomus mosseage, Glomus clarum, Glomus monosporus, Glomus deserticola, Glomus brasilianum, Glomus etunicatum and Gigaspora margarita*) AMF that include 23,5 % total living organisms were applied at 0 (control), 10 g/l and 20 g/l concentrations. The cuttings have been kept in these environments for approximately 3 months, shoots have been formed from cuttings and their roots and leaves were used for the purpose of conducting the analyses.

Analyses on root and shoot development

Rooting percentage

Among the plants subjected to various applications roots were counted and rooting percentage were thus determined as percentage (%).

Shoot weight

Through the use of a precision balance with 0.001 g sensitivity, shoot weights were determined as weight (g).

Shoot length

Through the use of a ruler, shoots lengths were determined as length (cm).

Analyses on biochemical changes

Chlorophyll analysis

Chlorophyll analyses were carried out in accordance with Witham et al. [13]. 0.5 g fresh leaf sample was extracted with 80% acetone and readings at 645 and 663 wavelengths were taken in spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll amounts were calculated in mg/g as per the formulas presented below.

Chlorophyll a (mg/g) = 12.7 x (A 663-2.69) x A 645 x V/1000xW
Chlorophyll b (mg/g) = 22.91 x (A 645-4.68) x A 663 x V/1000xW
Total chlorophyll (mg/g) = chlorophyll a + chlorophyll b
V = Extract volume (ml), W = Leaf weight (g), A = Absorbance value

Soluble sugar analysis

Soluble sugar analysis was carried out according to Robyt and White [14]. According to this, samples were mixed with 80% methanol and boiled for 30 minutes at 70^{o}C. Afterwards, phenol and sulfuric acid were added to the cooled mixture and it was read at 640 nm in spectrophotometer. The soluble sugar concentration was determined from a standard glucose curve and calculated on a fresh weight basis (µmol/mg).

Total phenolic compound analysis

Leaves were separated, homogenized with 10 times the volume ethyl acetate containing 0.1% HCl and extracted with three repetitions. After the ethyl acetated portions that contain phenolic compounds were washed through sodium sulfate, it was evaporated in rotary evaporator. Obtained extracts were later used in phenolic compound analyses. Total phenolic compound amounts were analyzed in line with Singleton and Rossi [15] through the use of Folin Ciocalteu colorimetric method. Spectrophotometer readings were carried out at 765 nm wavelength, and total phenolic compound amounts were determined in as mg/g in terms of gallic acid by utilizing the standard gallic acid curve. Analyses were conducted with 3 repetitions.

Statistical analysis

The data used in the study belong to three rootstocks (110 R, 41 B M.G. and Kober 5 BB), two concentrations and control groups. Tests were conducted with 3 repetitions with 15 plants included in each repetition. Data were subjected to analysis of variance with mean separation by Duncan's multiple comparisons test. Differences were considered statistically significant at the $p \leq 0.05$ levels. Statistical analysis was performed using packet programme of IBM SPSS Statistics 20.0

RESULTS

Results on root and shoot development

The analyses conducted for the purpose of determining root and shoot development in three different American grapevine rootstocks of 110 R, 41 B and 5 BB, all of which have different rooting percentage after mycorrhiza application are presented in Table 1. As it can be seen from examining Table 1, the effects of applying varying concentrations of AMF on American grapevine rootstock cuttings on root and shoot development has been largely variable. The rooting percentage, as the first parameter examined for growth and development, shows that applications of AMF in all genotypes generated substantially higher values than the control group. Particularly in the group where 20 g/l AMF was applied it was observed that the rooting percentage was 100 % both in 110 R and 5 BB genotypes (Table 1). In addition, in both application groups it was observed that the rooting percentage value of 41 B genotype increased by two times in comparison to the control group. Shoot weight values as an important indicator of growth and development did not result in the determination of any statistically significant difference in terms of genotypes or applications (Table 1).

Table 1. Effect of AMF on rooting percentage, shoot weight, and shoot length of Vitis genotypes

AMF		*Rooting percentage (%)*	*Shoot weight (g)*	*Shoot length (cm)*
110 R	*Control*	60 Ac*	2,90	13,47 b
	10 g	80 Ab	3,16	19,00 ab
	20 g	100 Aa	3,21	21,07 a
41 B	*Control*	40 Bc	3,95	17,67 b
	10 g	80 Bb	3,69	20,33 ab
	20 g	80 Ba	4,60	21,67 a
5 BB	*Control*	60 Ac	3,47	17,33 b
	10 g	80 Ab	3,11	18,00 ab
	20 g	100 Aa	4,62	23,00 a

*The capital letters show the difference among the genotypes and the lower letters show the difference among the AMF applications. The differences among the letters are important in p<0.05 level.

It was determined that applying AMF on three American grapevine rootstocks did not result in any statistically significant difference in shoot lengths. However, in terms of application groups it was determined that the longest shoots (23.00 cm) were on the 5 BB genotype subjected to 20 g application (Table 1). The shortest shoots (13.47 cm) were found in the control group of the 110 R rootstock.

Results on biochemical changes

Biochemical changes (chlorophyll, total phenolic compound and soluble sugar) triggered by the application of varying concentrations of AMF are presented herein below.

Effect of AMF on chlorophyll amount

The changes in chlorophyll a, chlorophyll b and total chlorophyll contents of the leaves triggered by subjecting three different American grapevine rootstock cuttings to AMF application are presented in Table 2. The table shows that statistically significant differences exist in between all genotypes and all application groups (p<0.05).

In data concerning the 110 R genotype, it was determined that the chlorophyll a, chlorophyll b and total chlorophyll contents were the lowest in the control group and higher in both application groups. Particularly, chlorophyll b content increased by approximately two times of the control group. In data concerning the chlorophyll content of the 41 B American grapevine rootstock, it was determined that as in the case of 110 R, particularly chlorophyll a content increased by two times with the application of AMF.

Table 2. Effect of AMF on chlorophyll content of Vitis genotypes

AMF		Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
110 R	*Control*	14.71 Bb*	5.92 Bb	20.63 Bb
	10 g	17.78 Ba	7.39 Ba	25.17 Ba
	20 g	20.54 Ba	10.37 Ba	30.91 Ba
41 B	*Control*	8.87 Cb	7.99 Bb	16.86 Cb
	10 g	16.62 Ca	6.40 Ba	23.02 Ca
	20 g	17.47 Ca	6.26 Ba	23.73 Ca
5 BB	*Control*	11.70 Ab	15.20 Ab	26.90 Ab
	10 g	21.32 Aa	21.50 Aa	42.82 Aa
	20 g	24.22 Aa	19.39 Aa	43.61 Aa

*The capital letters show the difference among the genotypes and the lower letters show the difference among the AMF applications. The differences among the letters are important in p<0.05 level.

In the chlorophyll a values of 5 BB rootstock, it was determined that in consequence of 20 g AMF application the chlorophyll a content of the leaves reached substantially high levels (24.22 mg/g) (Table 2) and that similarly with the cases of the other genotypes this level was more than twice the level of chlorophyll a in control.

In data concerning with chlorophyll a and total chlorophyll amounts, it was determined that the genotype 5 BB had the highest content in terms of both parameters, that this genotype was followed by 110 R and that the lowest values in this terms belonged to the 41 B genotype (Table 2). In terms of AMF applications, it was determined that these values were lowest in the control group and found out to be higher in both of the application groups. Also the highest content was found in 5 BB genotype, followed by 110 R and then by 41 B in the total chlorophyll content. Regarding the statistical analyses among all genotypes and all application groups, it was determined that the 5 BB rootstock had higher chlorophyll content than the other two American grapevine rootstocks. As for the two different AMF applications, although no statistically significant difference was found between them, they both resulted in higher chlorophyll amounts than the amounts in the control group.

Effect of AMF on soluble sugar content

Soluble sugars, particularly sucrose, glucose and fructose are compounds that play critic roles in plant metabolism. Soluble sugar contents of leaves were determined and the values are presented in Table 3. The effects of mycorrhizal fungi applications on soluble sugar

contents are presented, shows that 20 g AMF application increased the soluble sugar contents of the leaves to the highest levels. In addition, it was determined that the genotype 110 R embodied soluble sugars at a higher rate than the other two genotypes. For all genotypes used in the study, it was determined that the lowest soluble sugar contents belonged to the control groups.

Table 3. Effect of AMF on soluble sugar content of Vitis genotypes

AMF	*Soluble sugar* (µmol/mg)		
	110 R	***41 B***	***5 BB***
Control	7.00 Ac*	6.86 Bc	7.31 Bc
10 g	11.68 Ab	5.40 Bb	10.11 Bb
20 g	12.10 Aa	13.92 Ba	9.90 Ba

*The capital letters show the difference among the genotypes and the lower letters show the difference among the AMF applications. The differences among the letters are important in $p<0.05$ level.

Effect of AMF on total phenolic compound content

The total phenolic compound contents of leaves by AMF application are presented in Table 4. In Table 4 where the changes exhibited by total phenolic compounds as a result of AMF applications shows that the phenolic compound content of both application groups significantly increased in comparison to the control group of all genotypes.

Table 4. Effect of AMF on total phenolic compound content of Vitis genotypes

AMF	*Total phenolic compound* (mg/g)		
	110 R	***41 B***	***5 BB***
Control	0.42 Bb*	0.88 Ab	0.79 Bb
10 g	2.02 Ba	1.06 Aa	1.20 Ba
20 g	1.02 Ba	2.77 Aa	1.43 Ba

*The capital letters show the difference among the genotypes and the lower letters show the difference among the AMF applications. The differences among the letters are important in $p<0.05$ level.

It is observed that among all the genotypes used in the study, the phenolic compound content of 41 B reached higher levels than those reached by the other two American grapevine rootstocks. As a matter of fact, it was observed that the 0.88 mg/g total phenolic compound content of the 41 B genotype increased approximately three times and reached the highest value of 2.77 mg/g in consequence of 20 g/l AMF application.

DISCUSSION

Through the evaluation the findings obtained from the study together in terms of growth and development, it was determined that mycorrhizal applications promote vegetative development of grapevine rootstocks in many aspects. The presence of a significant relation between vegetative development and AMF was reported also in some studies previously conducted on this topic [16,17,18,19,20].

Also Podilla and Douds [21] stated that AMF play a significant role in plant development and growth, and that the main reason for this is the fact that AMF support the absorption of some nutrition elements and particularly phosphorus. Also in studies conducted previously in this area, it was reported that mycorrhizal plants contain more phosphorus than nonmycorrhizal plants [22,23,24,25,26,27] and that the photosynthesis rate of mycorrhizal plants is higher [28].

It is known that phosphorus has an important role as an energy carrier in photosynthesis. Thus, by promoting phosphor absorption, AMF enhance photosynthesis activity [29]. It is reported that the levels of some hormones such as cytokinin and gibberellin also increase in plants subjected to AMF, that particularly the increase in cytokinins promote the transfer of the ions that enable stoma opening and increase photosynthesis rate by regulating chlorophyll levels [30,31].

Mycorrhizal fungi have a significant role in protecting plants against biotic and abiotic stress conditions. It is believed that this is a result of the increases that take place in roots in some compounds such as arginine and isoflavonoid [12] and in some hormones such as cytokinin and gibberellin [32] as a reaction to the penetration of roots by mycorrhizal fungi. It is known that plants synthesize isoflavon and other flavonoids to protect themselves against several stress conditions such as damages [33,34], low temperature and inadequate nutrition [35]. In a study conducted by Morandi et al. [36] it was reported that mycorrhizas trigger the increase of some phytoalexins in soybean roots and that due to the antimicrobial characteristics of these compounds conditions preventing the growth of pathogens are established in the mycorrhizosphere. It is also reported that in consequence of mycorrhizal activity in cowpea and soybean isoflavonoid compounds similar to phytoalexine increase and that these compounds particularly affect the occurrence of wilt disease and the severity of the disease [37]. In another study Rabie [38] examined the effects of the inoculation of *Rhizobium leguminosarum* and the mycorrhizal fungi *Glomus mosseae* on plant development and the effects on *Botrytis fabae* as the chocolate spot disease in broad bean. As a result of the extraction of total phenolic acids and spectrophotometric analyses, the author reported that while the phenoic acid content in plants subjected to only pathogen application was 6.90%, the same reached 30.50% in plants subjected to dual symbiont application and disease inoculation. This clearly exhibits the relationship between phenolic compounds and plant diseases.

In the study it was determined that soluble sugars increased with AMF applications. Soluble sugars do not only act on the metabolic activities of the cells and structural components, but also as a signal regulators in processes related plant growth and development [39,40,41]. Sugars are in a complex relation with stress pathways regulating the plant's reaction against varying stress conditions [42,43]. It is known that soluble sugars are also effective in increasing freezing tolerance of plants. It was determined that, particularly in plants that are gradually exposed to low temperatures soluble sugars take place in this process by acting as osmoprotectants. The presence of the relation between mycorrhizal fungi and soluble sugars was demonstrated in studies conducted in this area. Graham [44] reported that the sucrose, reducing sugars (fructose, α glucose, β glucose) and total sugar contents in mycorrhizal plants are generally higher than those in control plants and that there is a positive relation between phosphorus concentration and sugar content. With the consideration that phosphorus also is effective in starch synthesis from glucose [45] and that AMF promote phosphorus absorption, this interaction can be better understood. Also Porcel and Ruiz Lozano [11] stated that sugar contents in mycorrhizal

plants are higher than the sugar contents of nonmycorrhizal plants.

In the conclusion of the study it was demonstrated that AMF are very important in shoot and root development and biochemical properties of American grapevine rootstocks. It is believed that these properties of AMF will have a great potential in viticulture industry. In addition, utilization of AMF in establishing new vineyards will ensure the enhancement of the yield and quality of grapevine by supporting the absorption of nutritional elements and also will be effective in preventing losses by enhancing the tolerance against varying stress conditions.

Acknowledgement

This study was supported by the Department of Agricultural Biotechnology, Suleyman Demirel University (TURKEY). The authors are grateful to Prof. Dr. N. GOKTURK BAYDAR for providing the laboratory facilities. We are thankful to Egirdir Fruit Research Station for providing the plant material and Bioglobal Company for providing the AMF preparations.

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