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Content

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Research Article

EFFECT OF DIFFERENT STORING TIMES ON GRAFT SUCCESS IN BENCH GRAFTED WALNUT PLANTS

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ABSTRACT. This study aimed to determine how long grafted walnut plants can be stored before transplanting them to the field. The experiment was conducted between 2012 and 2013 at Black Sea Agricultural Research Institute of Samsun, Turkey. One-year old seedlings of J. regia and scion woods of 'Chandler' cultivar were used as plant materials. The plants were grafted with whip-tongue, chip budding and Mr. Cherny grafting methods. In the study, two different storing treatments (heat & cold room treatment and cold room treatment) and four storing times (3, 6, 9 and 12 weeks) were tested. Graft success (%) was determined at the end of the vegetation period. The study was established with the experimental design of split - plots in random blocks with 3 replications. In each replication 18 plants were used. The highest graft success was obtained from whip-tongue grafted plants that subjected to the heat & cold room treatment and stored for 9 weeks by 83.3 % in 2013. Besides, it is determined that bench grafted walnut plants can be stored for 12 weeks after grafting. However, the rate of graft success decreases due to the increase in storing time.

Keywords: *Callus formation, J. regia, planting time, temperature*

INTRODUCTION

Walnut can be propagated by vegetative or generative methods. In the generative method, propagation material is seed. However, the biggest disadvantage of propagation by seed is the new plant can be different than the mother plant. Due to this disadvantage of generative propagation, vegetative propagation methods are commonly used for plant propagation. Among vegetative propagation methods, grafting is mostly preferred. Grafting of walnut requires more care than other fruit types [1, 2, 3, 4, 5, 6, 7]. Grafting success of walnut is affected by multiple factors such as cultivar, quality of the scion wood and rootstock, xylem exudation, collection time of the scion wood, grafting time and method, air temperature and relative humidity before and after grafting [1, 5, 8, 9, 10, 11]. Amongst them, the temperature is one of the major factors affect graft success of walnut. Many researchers reported that walnut does not form any callus below 20°C and the optimum temperature for callus formation is between 25 and 27°C [10, 12, 13, 14, 15]. This requirement for callus formation limits the grafting period for a short time. In ecologically unsuitable conditions, to extend the grafting period, grafting is performed in controlled indoor conditions [16].

There are two different indoor grafting applications. The first method is called as "Hot callusing at grafting point" method, which only heats graft union [2, 14, 16, 17, 18, 19],

the second method is called as “Classical method” that heats whole of the room to 25-28°C [20, 21, 22, 23]. In these two methods, most of the researchers transfer grafted plants into the greenhouse or open field immediately after graft healing. In the open field, unsuitable ecological conditions can cause graft failures. On the other hand, in the greenhouse, grafted plants should be potted and then should transferred to the open field. According to the pot size, potted plants generally have limited root area. So, it affects plant quality [24]. To overcome this problem grafted plants should be stored in proper condition and then should be transferred into the open field.

This study was planned to find out how long indoor grafted walnut plants can be stored before planting them into the open field.

MATERIALS AND METHODS

Material

This study was carried out between 2012 and 2013 at Black Sea Agricultural Research Institute in Samsun, Turkey. In the study, ‘Chandler’ cultivar was used as scion and one-year old Juglans regia seedlings as rootstocks. The scion woods were collected in January and they were stored at 4°C until grafting date.

Methods

Graftings were performed as bench grafting on 20-22 February in both years. Whip-tongue, chip budding, and Mr. Cherny grafting methods were used in the study. In Mr. Cherny grafting method a plastic material was placed between scion and rootstock (Fig. 1).

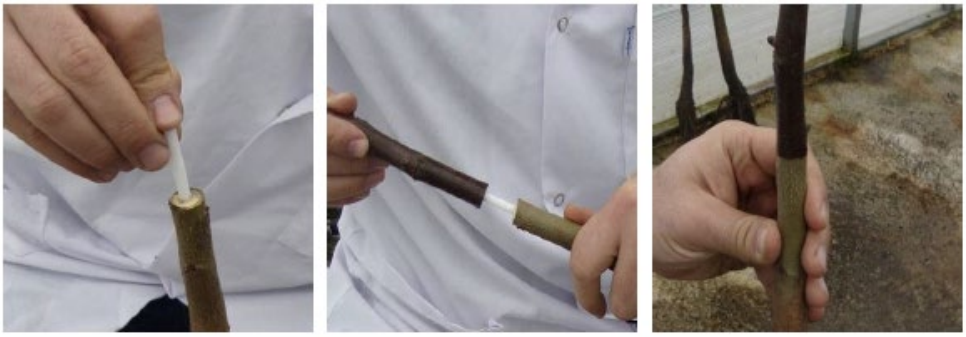

Fig. 1. *Application steps of Mr. Cherny grafting method*

Scion and rootstock were wrapped with plastic tape to hold them together. As an isolation material, water-based white paint was applied to the tip of the scion wood in whip-tongue and Mr. Cherny method and the tip of rootstocks in the chip budding method. In both years, graft union and scion woods were wrapped with parafilm.

Storing treatments

In 2012, grafted plants were put into wooden boxes as horizontally and they were completely covered with wet perlite. The top of the boxes was covered with polyethylene bags to avoid loss in moisture from the perlite (Fig. 2). On the other hand, in 2013 grafted plants were planted in plastic bags only their roots were covered with wet perlite (Fig. 3).

Fig. 2. *Storing of grafted walnut plants in a wooden box covered by polyethylene bag in 2012*

Fig. 3. *Storing of grafted walnut plants in perlite at the heat & cold and cold room in 2013*

Indoor grafted plants were subjected to two different storing treatments. In the first treatment, grafted plants were stored in a climatic room (24-27°C air temperature) for three weeks then they were transferred into a cold room (2-4°C temperature and 90 % humidity) and stored them until planting date (heat & cold room). In 2013, some of the grafted plants started to sprout from the scion wood. So, before transfer to the cold room whole of the plant was covered with slightly wet perlite within plastic bags. In the second treatment, grafted plants were stored in the cold room (2-4°C temperature and 90 % humidity) until the planting date (cold room). Air temperature and relative humidity of

the rooms were recorded by a data logger (KIMO 310). The temperature of the perlite medium was measured by a soil thermometer (measuring range -20°C to +50°C).

Storing times of grafted plants

After storing treatments, different storing times (about 3, 6, 9 and 12 weeks) were tested. For this aim, in 2012 and 2013 grafted plants were planted to the open field on 15 March, 5 April, 25 April and 15 May (3, 6, 9 and 12 weeks storing time, respectively). Grafted plants were planted in the open field by 20 x 150 cm spacing.

Study Design and Data Analyses

Graft success (%) was determined based on the number of grafts that survived at the end of the vegetation period. The study was established with the experimental design of split - plots in random blocks with 3 replications. In each replication 18 plants were used. 'JMP 17' statistic program was used to evaluate the data. $\sqrt{(x+1)}$ transformation was applied to the data due to 0% values [25]. The significance level of the differences between the means was determined by Duncan Multiple Range Test ($p \leq 0.05$).

RESULTS AND DISCUSSION

In 2012, graft success was ranged between 0 to 62.5 % according to the grafting method, storing treatment and storing time (Table 1). The best graft success was obtained from the plants propagated by chip budding method which were stored in the cold room for 3, 6 and 9 weeks (54.2 %, 62.5 %, and 58,7 % respectively) (Table 1).

Table 1. *Effect of storing treatments x grafting methods x storing time interaction on graft success (%) in 2012*

Storing Treatments	Grafting Methods	Storing Time				Mean
		3 weeks	6 weeks	9 weeks	12 weeks	
Heat & cold	Whip-tongue	9.4 de*	8.3 de	15.6 c	14.6 cd	**12.0 C****
	Chip budding	9.4 de	6.3 e	15.6 c	2.1 f	**8.3 C**
	Mr. Cherny	2.1 f	2.1 f	0.0 g	0.0 g	**1.0 D**
Cold	Whip-tongue	18.8 c	37.5 b	37.5 b	10.4 c-e	**26.0 B**
	Chip budding	54.2 a	62.5 a	58.7 a	2.1 f	**44.3 A**
	Mr. Cherny	0.0 g	0.0 g	0.0 g	0.0 g	**0.0 D**

* Difference between the means of storing treatments x grafting methods x storing time interaction shown in the same column with the same lower case is not statistically significant ($P \leq 0.05$).

** Difference between the means of storing treatments x grafting methods interaction shown in the same column with the same capital letter is not statistically significant ($P \leq 0.05$).

According to grafting methods chip budding (26.3 %) and whip-tongue (19.0 %) grafting methods gave the best results (Table 2). Storing times statistically affected graft success. The worst result was obtained from 12 weeks of storage.

Table 2. *Effect of grafting methods x storing time interaction on graft success (%) in 2012*

Grafting Methods	Storing Time				Mean
	3 weeks	6 weeks	9 weeks	12 weeks	
Whip-tongue	14.1 c*	22.9 b	26.6 ab	12.5 c	**19.0 A*** **
Chip budding	31.8 ab	34.4 ab	37.0 a	2.1 d	**26.3 A**
Mr. Cherny	1.0 de	1.0 de	0.0 e	0.0 e	**0.5 B**
Mean	**15.6 A****	**19.4 A**	**21.2 A**	**4.9 B**	

* Difference between the means of grafting methods x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).
** The difference between the mean of the grafting methods indicated by the same capital letter in the same line is not statistically significant (P≤0.05).
*** The difference between the mean of the storing times indicated by the same capital letter in the same column is not statistically significant (P≤0.05).

Contrast to the literature the plants subjected to cold room treatment had better graft success than heat & cold room treatment (Table 3).

Table 3. *Effect of storing treatments x storing time interaction on graft success (%) in 2012*

Storing Treatments	Storing Time				Mean
	3 weeks	6 weeks	9 weeks	12 weeks	
Heat & cold	7.0 c*	5.6 cd	10.4 c	5.6 de	**7.2 B****
Cold	24.3 b	33.3 a	31.9 a	4.2 e	**38.7 A**

* Difference between the means of storing treatments x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).
** The difference between the mean of the storing treatments indicated by the same capital letter in the same column is not statistically significant (P≤0.05).

As mentioned before, in 2012 after grafting, the whole of the plant was covered with perlite and stored in the wooden boxes until planting date. Perlite can hold 3–4 times its weight in water [26]. Perlite is a good material to keep scion woods or seeds alive in the cold room. However, in this case, high moisture level and temperature (about 24-27 °C) could have been damaged the graft area. Also, the graft area could be damaged mechanically when removing plants from the wooden boxes. Due to these negative results, in 2013 plants roots were only covered with wet perlite and placed vertically. Similar to the results, Akca et al. [27] obtained higher graft success (32.5 %) by using poplar sawdust as a cover material than perlite (20 %). Dehgan et al. [13] compared the effect of perlite and sawdust as a cover material on graft success. The sawdust had nearly two times better graft success compared to perlite.

In 2013, graft success was ranged between 0 to 83.3 % according to the grafting method, storing treatment and storing time (Table 4). The highest graft success was obtained from whip-tongue grafted plants that subjected to the heat & cold room treatment and stored for 9 weeks by 83.3 %.

Table 4. *Effect of storing treatments x grafting methods x storing time interaction on graft success (%) in 2013*

Storing Treatments	Grafting Methods	Storing Time				Mean
		3 weeks	6 weeks	9 weeks	12 weeks	
Heat & cold	Whip-tongue	77.1 ab*	68.8 a-c	83.3 a	62.5 a-c	**72.9 A****
	Chip budding	39.6 ef	54.2 cd	33.3 ef	29.2 fg	**39.1 B**
	Mr. Cherny	0.0 k	4.2 ı	0.0 k	2.1 ı	**1.6 C**
Cold	Whip-tongue	62.5 a-c	43.8 de	14.6 h	18.8 gh	**34.9 B**
	Chip budding	2.1 ı	0.0 k	0.0 k	0.0 k	**0.5 C**
	Mr. Cherny	0.0 k	0.0 k	0.0 k	0.0 k	**0.0 C**

* Difference between the means of storing treatments x grafting methods x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).
** Difference between the means of storing treatments x grafting methods interaction shown in the same column with the same capital letter is not statistically significant (P≤0.05).

In this year, the highest graft success was obtained from the whip-tongue method by 53.9 % (Table 5). Graft success also changed according to the storing time. The highest graft success was obtained from 3 and 6 weeks stored plants by 30.2 % and 28.5 % respectively (Table 5).

Table 5. *Effect of grafting methods x storing time interaction on graft success (%) in 2013*

Grafting Methods	Storing Time				Mean
	3 weeks	6 weeks	9 weeks	12 weeks	
Whip-tongue	69.8 a*	56.3 b	49.0 bc	40.6 c	**53.9 A*****
Chip budding	20.8 e	27.1 de	16.7 ef	14.6 f	**19.8 B**
Mr. Cherny	0.0 h	2.1 g	0.0 h	1.0 gh	**0.8 C**
Mean	**30.2 A****	**28.5 A**	**21.9 B**	**18.8 B**	

* Difference between the means of grafting methods x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).
** The difference between the mean of the grafting methods indicated by the same capital letter in the same line is not statistically significant (P≤0.05).
*** The difference between the mean of the storing times indicated by the same capital letter in the same column is not statistically significant (P≤0.05).

This year, better graft success results were obtained from heat & cold room treatment than cold room treatment. In heat & cold room treatment graft success was determined as 37.9 % and in the cold room as 11.8 % (Table 6). In cold room treatment except 3 weeks stored plants propagated by chip budding method, only graft success was obtained from the whip-tongue grafting method. However, graft success was decreased due to the increase in storing time (Table 4).

Table 6. *Effect of storing treatments x storing time interaction on graft success (%) in 2013*

Storing Treatments	Storing Time				Mean
	3 weeks	6 weeks	9 weeks	12 weeks	
Heat & cold	38.9 ab*	42.4 a	38.9 ab	31.3 b	**37.9 A****
Cold	21.5 c	14.6 d	4.9 e	6.3 e	**11.8 B**

* Difference between the means of storing treatments x storing time interaction shown in the same column with the same lower case is not statistically significant (P≤0.05).

** The difference between the mean of the storing treatments indicated by the same capital letter in the same column is not statistically significant (P≤0.05).

In 2013, because of the negative effect of perlite in the graft area, only roots were covered with perlite. This led the graft area to be subjected to air flow in the cold room. Reil et al. [28], Şen [29] and Hartmann et al. [10] suggested that walnut need high temperature (25-27 °C) for graft formation. Also, it has been reported that no callus formation was observed on the plants which were subjected 10 °C and 16 °C for three weeks [15]. In this period, it is important to keep scion woods alive before callus formation completed [11, 15]. This can also explain the low graft success on chip budding and Mr. Cherny grafting methods. In these methods, the graft formation area is smaller than the whip-tongue grafting method. So, they could be much more affected than cold compared to the whip-tongue grafting method. On the other hand, in heat & cold room treatment, before the transfer to the cold room, plants were covered with perlite. This could help to prevent air flow to damage the graft area.

Amongst grafting methods, Mr. Cherny grafting method gave the worst results in both years. Akyüz and Serdar [24] also indicated that they had low graft success with Mr. Cherny grafting method. In 2012 statistically, there was no difference between chip budding and whip-tongue grafting methods on graft success. In 2013 the best graft success was obtained from the whip-tongue grafting method. Also, similar results were obtained from different studies that compared chip budding and whip-tongue grafting methods [11, 21, 30].

In heat & cold room treatment the climatic room's temperature was set between 24-27 °C for three weeks (classic method). On the other hand, Achim and Botu [30] compared the classical method with hot callusing at the grafting point. They had better graft success with hot callusing at grafting point compared to the classic method (81.3 % and 59.6 % respectively). Also, in another study effect of hot callusing at graft point on graft success was tested. In the first-year, graft success was determined as 80.9 % and in the second year as 92.4 % [18].

CONCLUSION

As a conclusion, the highest graft success was obtained from whip-tongue grafted plants which subjected to the heat & cold room treatment and stored for 9 weeks by 83.3 % in 2013. Besides, grafted walnut plants can be stored for 12 weeks after grafting. However, the rate of graft success decreases due to the increase in storing time. Graft success can be increased by heating treatment. If there is no possibility for heating, grafts could be stored directly in the room conditions or cold room. In this case, graft formation starts after planting to open field. In both cases, the viability of graft unions or scion shoots should be maintained. For this aim, covering the whole of the grafted plant with

perlite is not a desirable method due to high temperature and moisture in a heated room or mechanical damage when removing plants from boxes. More studies should be done with hot callusing at graft point. Whip-tongue grafting method had better graft success compared to other methods. However, this method requires more care than other methods. So further studies should be done with other grafting methods.

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Research Article

THE EFFECTS OF THE MYCORRHIZA ON PLANT GROWTH DURING ACCLIMATIZATION OF SOME *IN VITRO* GROWN SWEET CHERRY ROOTSTOCKS

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ABSTRACT. This study was conducted to investigate the effects of *Glomus mosseae* and *G. fasciculatum* on plant growth during acclimatization of micropropagated sweet cherry rootstocks. In order to determine the effects of mycorrhizal inoculation and growth media on plant growth, shoot length, and dry weight of roots and shoots were analyzed. The leaf content was significantly increased by mycorrhizal inoculation. The total root length and percentage of infected roots were investigated at the end of the acclimatization. As a result, survival rate of rootstock plantlets was not affected by mycorrhizal inoculation. The effect of inoculation was found significant on nutrient uptake and tissue P content. The results showed that mycorrhizal inoculation may be used at the *in vitro* rooting stage for better acclimatization. Mycorrhizae inoculated rootstocks grow better and increase Zn and P uptake.

Keywords: *Sweet cherry (Prunus avium L.) rootstocks, micropropagation, acclimatization, mycorrhizal inoculation, Zn, P uptake*

INTRODUCTION

After being worked on for thousands of years, agricultural lands have been exhausted due to the same old cultivation methods intensively practiced by the greedy human beings. Poor management practices such as over-fertilization and wrong pesticide applications have aggravated the productivity of the agricultural lands and have lead to lower production with higher inputs. Moreover, chemical applications by uneducated farmers have destroyed the natural balance of soil and upset the activity of the soil micro organisms. These problems have been more prominent by the end of the 20th century and alarmed farmers and researchers for solutions. New superior cultivars and organic farming have proved to be the feasible practices to remediate the problems. Researchers have shown that organic farming creates a well-balanced soil fauna and available nutrients both for the plant roots and for mycorrhiza fungi [1, 2, 3, 4, 5, 6]. Fungi involved in mycorrhizae associations exhibit the most common plant-microorganism symbiosis in nature. Botanically, mycorrhiza is the mutual relationship between the roots of higher plants and the soil fungi. Mycorrhizae fungi infect the root cortex and send out its hyphae (vesicular–arbuscular structures) into the cortex to become part of the inner root structure. Hyphae produce a network connecting the inside to outside of the plant and transferring nutrients from outside to inside and

carbohydrates from inside to outside [7]**.** Sustainability of the world population by the agricultural resources is of great concern as the population is expected to double by the end of the 21st century. This concern has led the developed countries to utilize new techniques and plant biotechnology to increase their agricultural yields. Plant propagation methods such as "plant tissue culture or micropropagation techniques" used in producing plants free of disease-causing agents are becoming more and more common. Micropropagation technique is a modern method to produce and also lets the fast-clonal propagation of plant species within a certain time [8, 9, 10]. By using the tissue culture or micropropagation method, disease-free plants expressing the desired characters can be produced in large amounts in a short period of time. Salamanca et al., [11] has demonstrated that AMF inoculation reduced the length of the micropropagated plant production cycle from 18 to 10 weeks. However, beside these advantages, the main problem in tissue culture is the acclimatization of the *in vitro* propagated plants to field environment. There are great losses when plants are transferred from the nearly 100 % humidity *in vitro* conditions to the field environment. The deaths are due to drying out which is caused by the fact that plants do not produce the water conserving cuticle layer under *in vitro* conditions. From the early stages of development, plant roots need the beneficial rhizosphere organisms, mainly the *Mycorrhizae* fungi [12, 13,14, 3, 15, 6, 5]. Rai, [16] indicated that arbuscular mycorrhizal fungi (AMF) improves bio priming of micropropagated plantlets and plays a significant role in ensuring the health of plantlets. Azcon-Aguilar et al. [17] reported that mycorrhiza formation appears to play a key role in favoring ex vitro development of micropropagated plants of avocado. A well-developed and strong root system aids the plant in water and nutrient uptake, and hence, improves the survival chances of plants which are produced by tissue culture methods. This research has analyzed the acclimatization techniques of cherry rootstocks propagated by tissue culture methods, and the effects of mycorrhizae in the acclimatization process. It has been reported [17, 18], inoculation of arbuscular mycorrhizal fungi (AMF) to the roots of micropropagated plantlets plays a beneficial role on their post-transplanting performance. Yadav et al. [19] indicated that an AM fungus during the initial period of the acclimatization phase has showed stimulatory effects for achieving better survival of micropropagated plantlets. Since period of micro propagated plantlets is short, for rhizosphere organism development it is sound to inoculate with mycorhizophere.

The goal of the research was to produce a well-developed and strong root system by using the symbiotic fungi and to overcome the difficulties in transferring the plants to field conditions and hence reducing losses. In addition, the stronger root systems will improve the absorption capacity of the plant supplying water and important nutrients such as phosphorus, and making the plant stronger against stress factors. Use of mycorrhizae as natural fertilizer is also an important goal as the chemical fertilizers are expensive and considered potentially harmful. In the light of these factors, the goal of this research is to improve the acclimation of cherry rootstocks to field conditions, and determining the optimum mycorrhizae and growth medium for the root system of the cherry rootstocks.

MATERIALS AND METHODS

The study included investigation effects of the mycorrhizae on plant growth during acclimatization of some micropropagated three cultivars of sweet cherry rootstocks. In

the experiment, all sweet cherry rootstocks (Gisela-5, Damil and Edabriz) were micropropagated and firstly acclimatization was made by the University of Çukurova, Faculty of Agriculture, and Department of Horticulture Laboratory for Plant Biotechnology**.** Also, mycorrhizal materials were supplied from University of Çukurova, Soil Science department which had used mycorrhizal species called *Glomus mossae* [19] and *Glomus fasciculatum* [21]**.**

Medium and Explants Establishment

In the experiment, Murashige and Skoog (MS) medium was used for micropropagation, growing and rooting [22]. The same procedure was used for shoot-tip culture. Shoot tip was isolated (0.1-0.5 mm) from shoots in a sterile bench and transferred into test tubes. Shoot tips taken to growth chamber with 16 h photoperiod (3000-4000 lux) at 26-28º C. Plants were transferred to new media per four weeks. After four weeks from *in vitro* rooting, the experiments were carried out greenhouse conditions.

Growth Substrata and Inoculums Properties

The plants were transferred into pots containing two types of growth substrates (GS);

- GS1; peat: perlite (1:1 v/v)
- GS2; Andesite: Soil: Compost (6:3:1 v/v).

The substrate was autoclaved at 121ºC for 2 h to use as growth substrate. Mycorrhizal inoculation was applied to plants when they were transferred from *in vitro* to *in vivo* same time. Control plants were transplanted into growth substrates without inoculum. Inoculum was obtained from cultures of *Glomus mosseae* and *G. fasciculatum* propagated on corn (*Zea mays*). Sand containing spores and infected root fragments were added to transplant substrates (1000 spores per plant).

Biomass Assessment and Nutrient Analysis

After three months, rooted plants were harvested and assayed for changes in biomass and root morphology. Roots were separated from the soil by washing with running and distilled water. Before drying the roots, small sub samples were taken for determination of root length and mycorrhizal infection using modified method from Koske and Gemma [23]. Washed root segments (1-2 cm) were cleared with 10% KOH for 30 min, rinsed with tap water, acidified in 5% HCl for 30 min and stained with trypan blue in lactic acid. Mycorrhizal colonization was determined using grid-line intersects method [24].

Shoot and root fresh and dry weights, survival rate, shoot height, and the number of leaves were recorded for each plant. Shoot and root fresh weights shoot and root dry weights were subsequently determined after drying at 65ºC for 48 h. Dry material from each pot was ground by Tema mill. Then 0.2 g of ground plant materials was ashed at 550ºC followed by dissolution in 3.3% HCl. After the digestion of the plant material, the concentration of P was determined by the Murphy and Riley, [25] method by using a spectrophotometer. The concentration of Fe, Zn, Cu and Mn was determined by atomic absorption spectrophotometer.

Experimental Design and Statistical Analysis

The experiments were analyzed with the MSTATC statistical programs [26] as Completely Randomized Designs with three replications and 10 plants for each replication and clones were used as covariate to control random variation. For all characteristics studied, the statistical significance of differences between means was determined using the Tukey-test.

RESULTS AND DISCUSSION

In order to observe the effects of the mycorrhizae and the growth substrate on the growth and development on the cherry rootstocks, the plants were grown for 12 weeks and then harvested. Although the findings indicated no significant effects of the mycorrhizal applications on the acclimatization of plants, the best results in both substrates (GS 1and GS 2) were obtained with *Glomus mosseae*. This application produced 89.82 % success with Gisela-5 cherry rootstocks.

Under the natural environment, majority of plants are colonized both by external and internal microorganisms; particularly such as beneficial bacteria and fungi, can improve plant performance under stress environments, and consequently enhance yield [27]. Other research carried out on field adaptation of indoor lab propagated plants support that mycorrhizal inoculation of the plants should be administered for higher survival rates. In addition, it is reported that the adaptation period is the most important step following the micro propagation techniques where the utilization of microorganisms might improve chances [28]. *In vitro* propagated plants are delicate and lacking vigor to survive the acclimatization shock with great losses observed frequently [29]. On the contrary, another research has reported that the infection was observed after the first 4 weeks, so that the inoculation did not have any effect on the survival rates of the micro propagated plants [30]. The same results were also observed in present research.

The effects of mycorrhizal applications and growth media on shoot length have been determined as well. Gisela-5 control plants showed greatest shoot growth (16.1 cm).

In this research, among the most important results, percentage of the infected root finding has been found. All investigation factors were significant and the highest infection rate was found *G. mosseae* application that belong Gisela-5 rootstock in GS-2 (% 89.82) (Fig. 1 and 2).

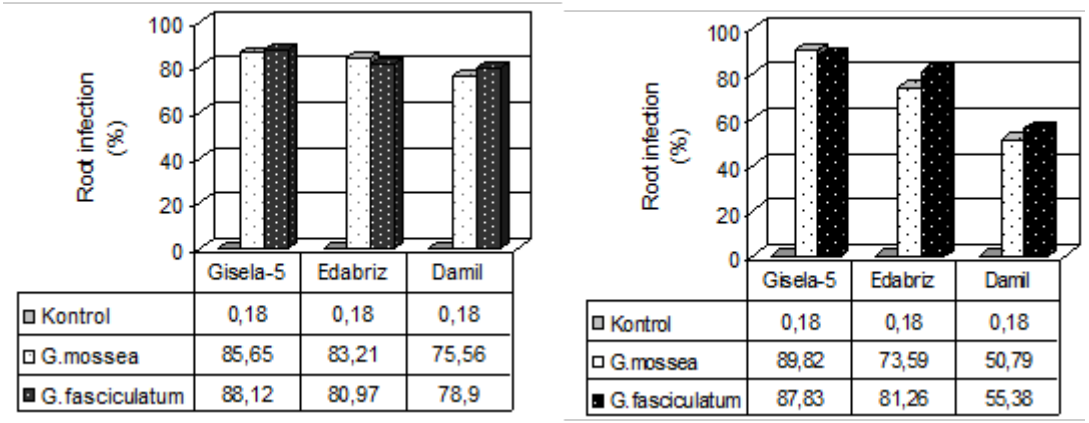

Fig. 1. *Cheery rootstocks percentage of the infected root finding grown on GS-1 and GS-2 (Respectively)*

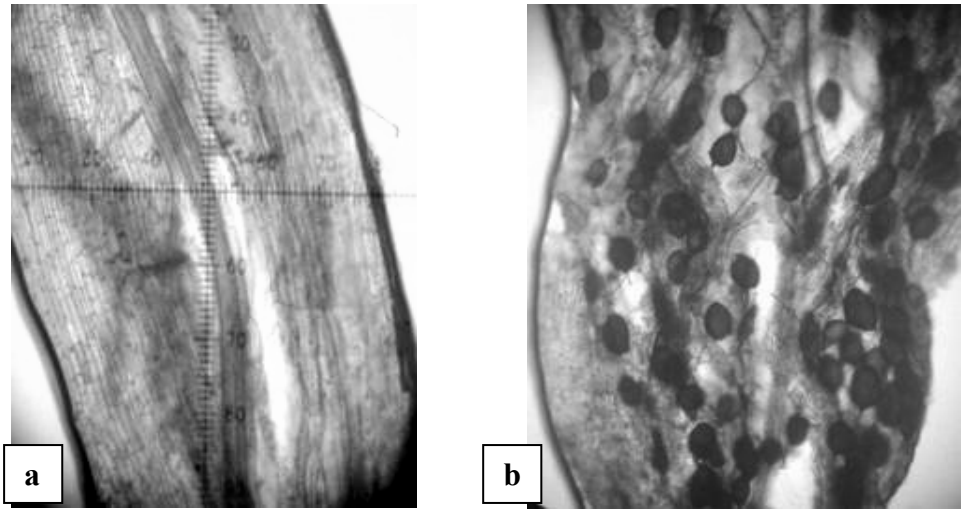

Fig. 2. a: *(-) non-mycorrhizal and* ***b:*** *(+) mycorrhizal root inoculation*

The effect of mycorrhizal application on total root length has been determined in order to show positive effects over the control plants. According to the shoot dry weights of cheery rootstocks, the highest shoot dry weights were determined by interactions of the variety+application+growth media. This rate was determined as 1.056 g by Gisela-5 control plants. Also, the type of GS-2 has been determined as the best media for shoot dry weights (Fig. 3).

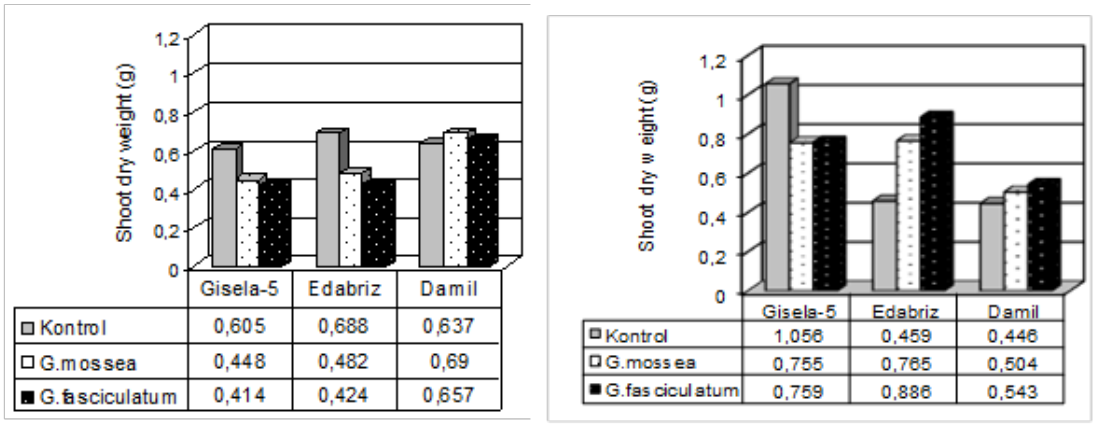

Fig. 3. *Shoot dry weights of Cheery rootstocks grown on GS-1 and GS-2 (Respectively)*

The highest root dry weights were determined by interactions of the variety + application+ growth media. This rate was determined as 0.88 g by Gisela-5 rootstocks. In generally for both growths medium non inoculated control plants produced high root dry weight. Also, the type of GS-2 has been determined as the best media for root dry weights (Fig. 4).

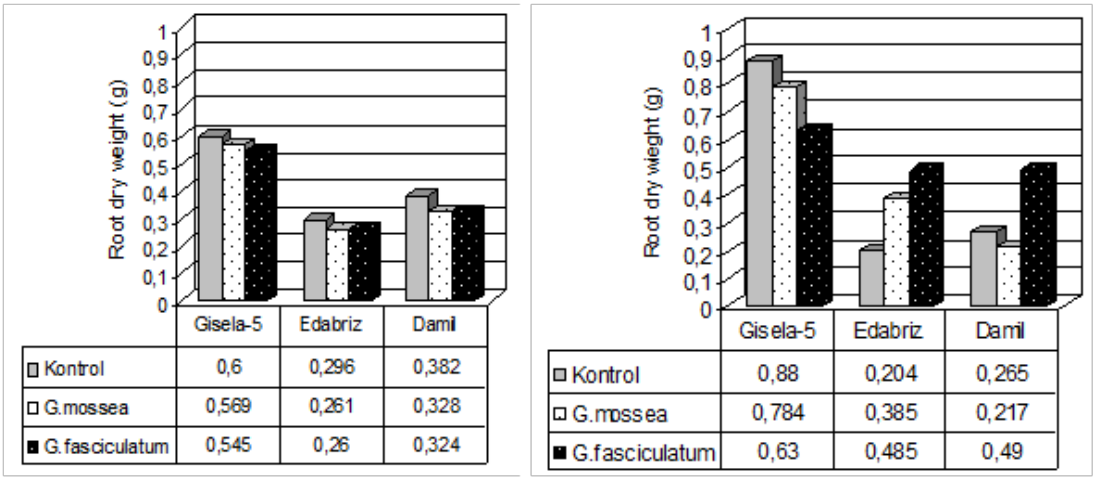

Fig. 4. *Root dry weights of Cheery rootstocks grown on GS-1 and GS-2 (Respectively)*

From these results it seems that the effects of isolates are different. Previously it has been reported that isolates of the fungi differ in their ability to benefit plants and this can be modified by both plant species and environmental condition [31]**.** Previously Hooker et al., [31] indicated that mycorrhizal inoculation usually resulting in root systems which are more branched and therefore likely to have a higher capacity for uptake of nutrient and water.

According to the leaf analysis, significant results have been obtained. The leaf content of P, Zn, Cu and Fe were tested and the highest iron (Fe) concentration was determined by interactions of the variety+application+growth media as 574.7 mg kg^{-1} with *Glomus fasciculatum* (Fig. 5).

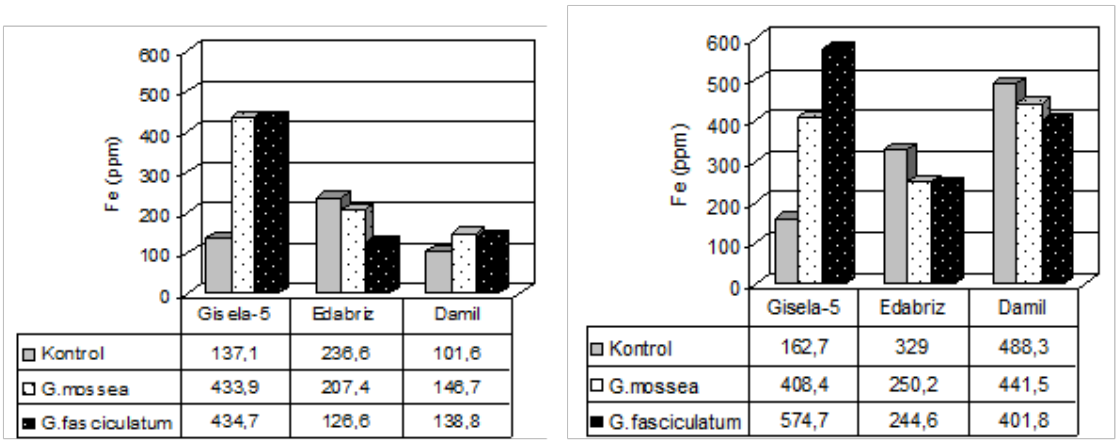

Fig. 5. *Cheery rootstocks Fe content (ppm) grown on GS-1 and GS-2 (Respectively)*

The leaf content of copper (Cu) has increased by mycorrhizal applications and the highest leaf content of Cu was obtained by interactions of the application + variety + growth media. This rate was determined as 22.71 mg kg^{-1} by Damil with *G. mosseae*. The type of GS-2 has been determined as the best media for leaf content of Cu (Fig. 6).

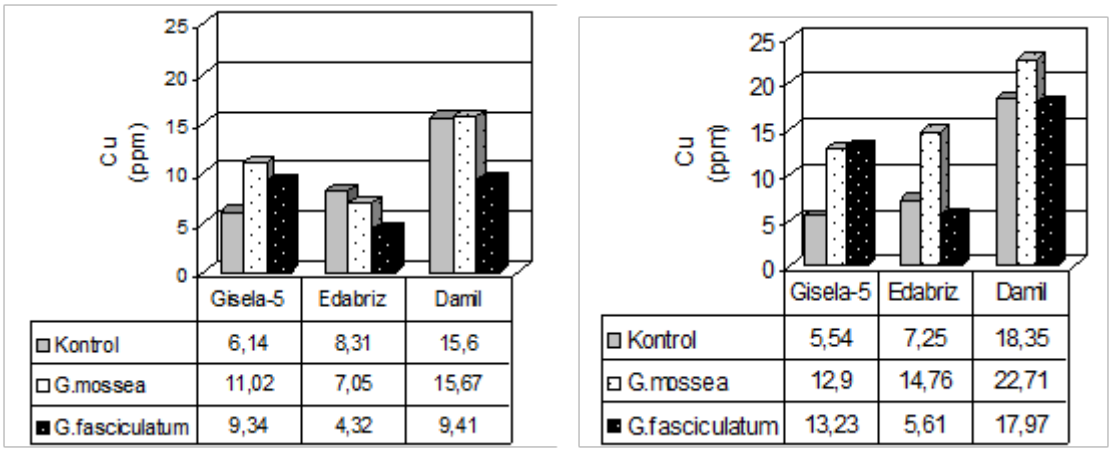

Fig. 6. *Cheery rootstocks Cu content (ppm) grown on GS-1 and GS-2 (Respectively)*

The leaf content of Zn has increased by mycorrhizal applications and the highest leaf content of Zn showed interactions of the variety+application+ growth media. G. *mosseae* more effective than *G. fasciculatum*. The primary effect of AM on their host plant is an increase in plant P and Zn uptake [32]. It seems that *G. mosseae* is the efficient inoculum for Gisela 5 and Edabriz rootstocks zinc uptake. This rate was 38.09 mg kg^{-1}. This result was obtained for the type of GS-1 (Fig. 7).

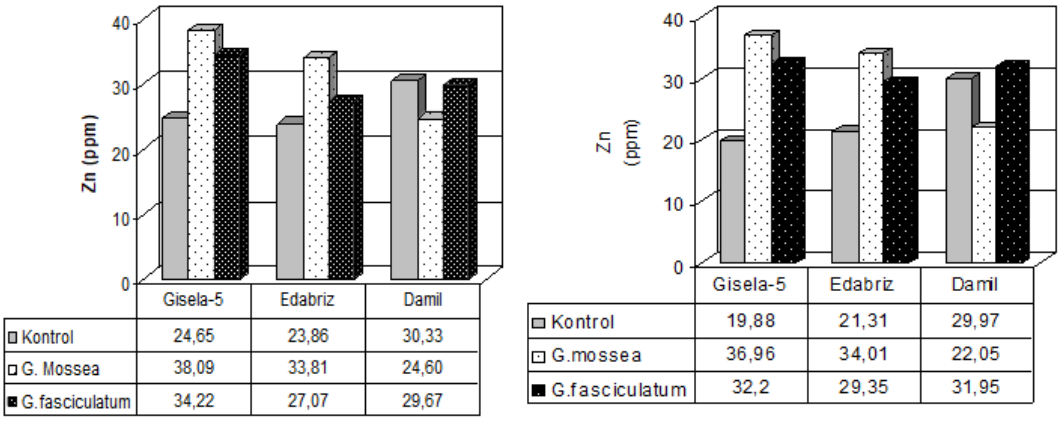

Fig. 7. *Cheery rootstocks Zn content grown on GS-1 and GS-2 (Respectively)*

Lastly, the effect of mycorrhizal applications on the leaf content of Phosphorus (P) has been showed to be double amount of concentration according to the control plants. From those varieties Edabriz showed the highest concentration. The other highest concentrations were determined on Gisela-5 and Damil. The type of GS-1 has been determined as the best media for leaf content of P. General appearance of P content is shown in *Fig. 8.* It seems that *G. fasciculatum* is the one of the efficient inoculums for P uptake. It has been reported the inoculation with AM in horticultural plants can improve growth by increasing the uptake of P, Zn and other minerals [33, 34]**.**

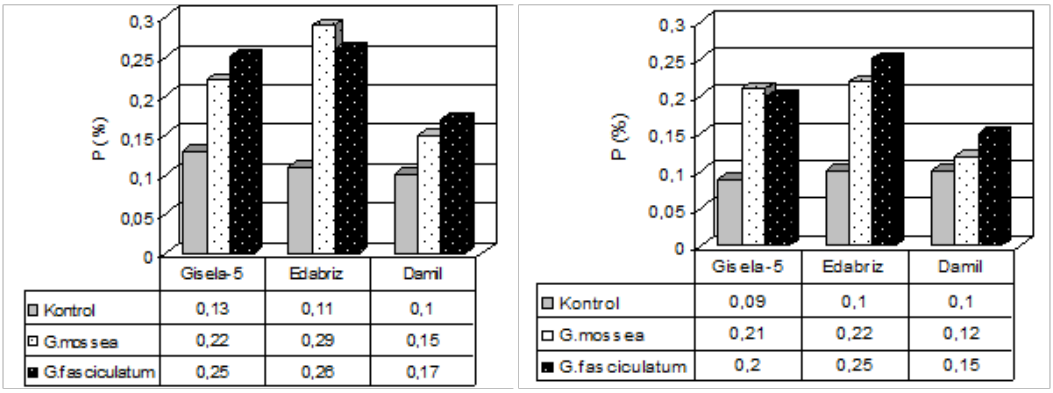

Fig. 8. *Cheery rootstocks P content (%) grown on GS-1 and GS-2 (Respectively)*

CONCLUSION

In recent years, the demands for micro propagated plants in the modern fruit cultivation have increased very much. Furthermore, before establishing of orchards, it is necessary to produce and use healthy plants, especially if the natural and non-chemical materials are not used for the healthy plant production, it can be very useful for the plant growth and human health. The results of this experiment indicated that micropropagated cherry rootstocks are significantly inoculated with mycorrhizal species under several growth media. It seems both inoculums are efficient mycorrhizal species for both cherry rootstocks and growth mediums. Results have shown that GS-2 has produced more dry weight and feed plant better than the GS-1. It was determined that the mycorrhizae affected sweet cherry rootstocks were very healthy and plant tissues are very rich in plant nutrients, and these results seemed to be in favour of the previous research. It is better to use early several mycorrhizae species with several levels of inoculum to find the best inoculum time and ratio of inoculum use for further research. The results are shown that AM species have significant effect on nutrient uptake of cherry rootstocks.

Acknowledgement. This study was supported by Çukurova University Scientific Research Projects (BAP) Office Directorate in 2002.

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Research Article

DETERMINATION OF THE GENETIC RESOURCES OF LOCAL GRAPES GENOTYPES (*Vitis vinifera* subsp. *sativa*) IN KAYSERI REGION

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ABSTRACT. Kayseri, which is the cradle of many civilizations, is one of the oldest and most ancient settlements of Anatolia retained its importance from past to present. Kayseri, which is folk songs written on behalf of it' s name; such as; Gesi Bağları, Erkilet Bağları, İncesu Bağları etc., is a city with a well-known vineyard background, famous for its vineyards and grapes. On the other hand, due to the fact that the studies on the collection of local grape genotypes in Kayseri remain very limited, the grape genetic potential of the region has not been determined. Especially in recent years, giving up the cultivation of local genotypes with commercial concerns, the increase of urbanization and the shift of this increase to vineyard areas, the cultivation of local grape genotypes of Kayseri is decreasing day by day. If these valuable gene resources are not protected, they will be lost in the following years. This study was carried out in the vineyards areas and home gardens in Kayseri province and its districts in 2018 and 23 different regions were investigated in the center, district, and village. At the end of the study, a total of 174 local genotypes known by local people and grown for many years were determined. In the collected genotypes, from the point of their local name, 55 different genotypes were determined. In addition, the local grape genotypes belonging to Kayseri region will be correctly reproduced as true to type and a core collection vineyard will be established and the loss of the vine genetic resources will be prevented with this study.

Keywords: *Genetic resource, grapes, grapevine, local genotypes, Kayseri*

INTRODUCTION

Plant genetic resources have been indispensable basic raw material due to their importance for food and agriculture since long time. Traditional and local varieties are very valuable resources; highly adapt to the ecological conditions of the region and remarkable resistance against pests and diseases. They carry many quality qualities such as taste, color and size [1]. Local varieties gain importance in the development of new varieties [2].

Turkey's defined as the collection of local grape varieties and ampelographic many studies have been made in the past to the present, such as; Adana region [3], Sivas region [4], Konya and Karaman regions [5], Southeastern Anatolia region [6], West Mediterranean region [7] and İncesu / Kayseri [8]. The most important work was done by Tekirdağ Viticulture Research Station in 1965, 'National Collection Vineyard' was established and morphological descriptions of varieties were made [9].

Kayseri region of Turkey has been a vineyard area since ancient times. Kayseri is a famous province for its vineyards and grapes such as Gesi Vineyards, Erkilet Vineyards, and Incesu Vineyards etc. There is a rich genetic diversity in Kayseri from the past to the present. The cultivation of local grape varieties belonging to Kayseri such as Dimrit, Buludu and Parmak, which are known and loved by the local people, is decreasing. Especially in recent years, migration from rural areas, economic reasons, and the shift of urbanization into vineyard areas, these valuable gene resources are decreasing day by day. If the existing local varieties are not protected, they will disappear in the following years.

In this study, the local grape genotypes belonging to Kayseri region will be correctly reproduced as true to type and a core collection vineyard will be established and the loss of the vine genetic resources will be prevented.

MATERIALS AND METHODS

Material

This study was carried out in the vineyards areas and home gardens in Kayseri province and its districts in 2017-2018. 23 different regions were investigated in the center, district, and village (Fig. 1).

Fig. 1. *Kayseri province and district map*

Method

Firstly, in line with the harvest schedule, interviews were made with the agricultural engineers in the provincial and district directorates of agriculture and with the local people on district and village basis. Regarding the received references, the growers and vineyard areas were visited and information was given about the study within the framework of the survey-level interviews.

The local grape genotypes in the vineyards representing the region were identified and the necessary investigations were made, and healthy and productive vines (Province plate no - Region name abbreviation - Genotype no) were labeled as their location.

RESULTS AND DISCUSSION

In this study, Incesu district which has wide vineyard area and grape production; Garipçe, Bedir, Hamurcu and Süksün regions are divided into 4 different regions. Other districts studied were Develi, Yeşilhisar, Yahyalı, Özvatan, Bünyan, Sarıoğlan, Pınarbaşı, Sarız, Talas, Hacılar and Tomarza districts and Yuvalı, Yüceyar, Erkilet, Gesi, Hisarcık, Kızıltepe, Eğribucak and Mimsin districts connected to central districts.

As a result of the screening studies, a total of 174 local genotypes were identified from different regions. Among these genotypes, 55 different genotypes were determined in terms of local name. The number of genotypes examined by region (Fig. 2) and their local names (Table 1) are presented below. Some local grape genotypes in the collection vineyard are presented in Fig. 3.

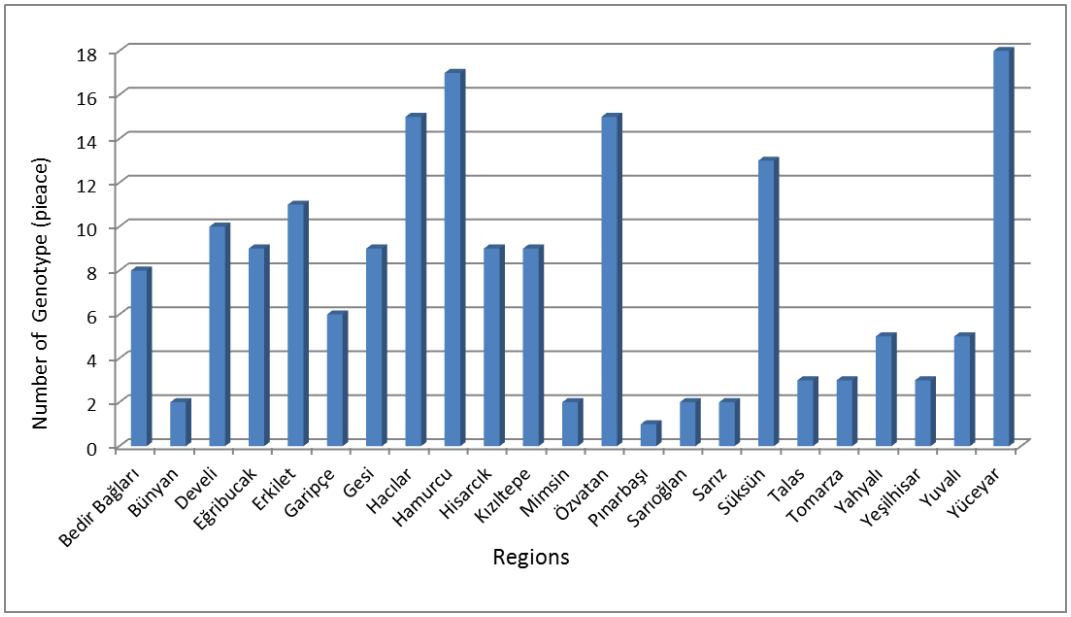

Fig. 2. *Distribution of local grape genotypes by regions*

Table 1. *Local grapes name in Kayseri*

Genotype No	Local Grape Name	Genotype No	Local Grape Name
1	Beyaz Buludu	**29**	Karabekir
2	Beyaz İrek	**30**	Kara burcu
3	Beyaz Keçimemesi	**31**	Karalık
4	Beyaz Şıralık	**32**	Kayseri Karası
5	Boz Geçemceği	**33**	Koç Taşı
6	Çakıltaşı	**34**	Kokulu Hevenk
7	Çavuş	**35**	Misket
8	Çekirdeksiz	**36**	Mor Buludu
9	Dana Boyu	**37**	Orun
10	Kara Hevek	**38**	Parmak İnce Kabuk

11	Deve Dişi	**39**	Parmak Siyah Buludu
12	Dökülgen	**40**	Parmak Üzüm
13	Dimrit	**41**	Parmak Yuvarlak Tane
14	Uzun Salkım	**42**	Pembe Çekirdeksiz
15	Eldaş	**43**	Pembe Dimrit
16	Gelin Yanağı	**44**	Sık Dimrit
17	Gemre	**45**	Siyah Çekirdeksiz
18	Göğcek	**46**	Siyah İrek
19	Gül Parmak	**47**	Siyah İri Tane
20	Gül Üzümü	**48**	Siyah Şıralık
21	Hevenklik Beyaz	**49**	Sungurlu Karanıdere
22	Irazakı	**50**	Şahabı
23	İri Beyaz Üzüm	**51**	Şireder
24	İstanbul Üzümü	**52**	Tavşan Kanı
25	Kara Buludu	**53**	Tilki Kuyruğu
26	Kara Burcu	**54**	Uzun Taneli Buludu
27	Kara Evrek	**55**	Yerli Dimrit
28	Kara Keçimemesi		

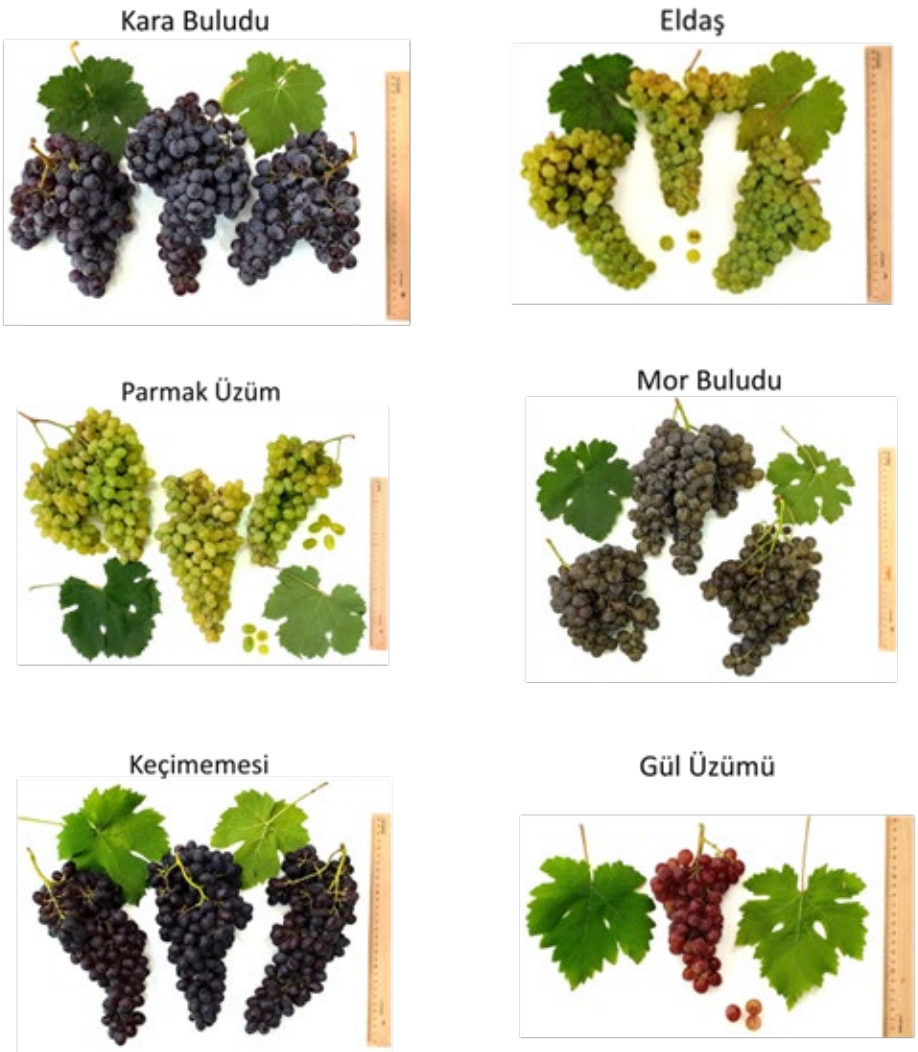

Fig. 3. *Some local grape genotypes from Kayseri in the Collection Vineyard*

In different regions of Turkey, to determine the grapevine genetic potential in many carried out studies, it is concluded that local grape varieties are valuable, important and conservation genetic resources. These studies were carried out in productive vineyards similar to our study, and grape varieties used for many years and grown locally were collected. Local grape varieties have been determined in the productive vineyards (Bineteti, Emiri, Hergifi, Heseni, Keşirte, Meyme, Zeynep, Sinciri, Şevkeye and Veledezine) in the central districts and villages of Siirt province [10]. Also, 9 local grape varieties (Pırtik Üzümü, At Memesi, Kişmiş Üzümü, Beyaz Üzüm, Kara Üzüm, Kabarcık, Beyaz At Memesi, Hatun Parmağı and Al Üzüm) in Olur district of Erzurum province [11]; 40 local varieties in Konya and Karaman provinces [5]; Kara [12] reported that 44 local grape genotypes in Tokat province; 29 local genotypes have been determined in İncesu district of Kayseri province by Kara [8]. 10 local grape varieties named Devegözü, Sık sarı, Siyah üzüm, Beyaz üzüm, Ak üzüm, Gelin üzümü, Kara erik, Sivri kara, Yediveren and Ballı have been identified in the Yuntdağı region of Manisa provience [13]. In the research carried out in the productive vineyards located in Gemerek district of Sivas province, 9 grape varieties named Karabekir, Göğcek, Dişieldaş, Kabaeldaş, Kehribar, Patlakkara, Gülüzümü, Memeüzümü and Dikkarabekir were identified [4]. 6 local grape varieties named Veyisoğlu, Kanlı Üzümü, Acıkara Üzüm, Pembe Çavuş, Kara Dirmit and Sarı Emin, were identified in Afyonkarahisar province [14]. In the Nevşehir region, 15 local genotypes named Emir, Dimrit, Çavuş ve Parmak üzüm, İsmailoğlu, Devedişi, Kayseri karası, Topak çavuş, Hacıoğlu siyahı, Ağın, Beyler, Çubuk siyahı, Çubuk beyazı, Horoz karası and Kalecik beyazı were identified [15].

As a result of our study, it has been observed that similar genotypes as named Dimrit, Parmak üzümü, Devedişi, Göğcek and Karabekir were determined in studies conducted in Nevşehir [15], Sivas [4] and İncesu [8].

CONCLUSION

At the end of this study, the collection vineyard was established within the Seyrani Faculty of Agriculture and the loss of local grapevine genetic resources was prevented.

In addition, with this study, it was provided to introduce and spread the quality local varieties that were abandoned in commercial concerns. This research will be a pioneer and guide for scientific viticulture studies to be conducted in Kayseri in the coming years. Morphological and molecular characteristics of local grape genotypes will be determined in later studies and similarities and differences will be revealed.

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Research Article

PHYTOCHEMICAL SCREENING AND ANTI-BACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF THE AERIAL PARTS OF *Atriplex halimus* L., FROM BISKRA (ALGERIA)

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ABSTRACT. The objectives assigned to the present study are the phytochemical screening of several secondary metabolites and the evaluation of the antibacterial activity of the methanolic extracts of the aerial parts of *Atriplex halimus* L. Phytochemical screening revealed the presence of substances with high therapeutic values (flavonoids, tannins, polyphenols, coumarin, etc.). The antibacterial activity of the extracts is carried out by the diffusion method on agar medium vis-à-vis sixteen bacterial strains, chosen according to the traditional use of this species in Algeria: *Staphylococcus* MRSA, *Staphylococcus aureus*, *Staphylococcus* ATCC 00, *Streptococcus* Sp, *Enterococci* Sp, *Enterococci feacalis* ATCC 12, *Salmonella, E. coli* ESBL +, *Klebsiell*a pn Marseille, *Kp*C+, *Kp* ETP R / IMP R, *Serratia* Sp, *Serratia environmental*, *Pseudomonas aerogenosa* ATCC 53, *Pseudomonas* VIM 2, *Bacillus*. The study shows a remarkable antibacterial activity against Gram+ bacteria compared to Gram- bacteria.

Keywords: *Atriplex halimus L., Biskra, aerial part, methanolic extract, antibacterial effect*

INTRODUCTION

Today, and despite the advances made in medicine, many people resort to plants for treatment, either because of inaccessibility to drugs prescribed by modern medicine, or because these plants have given very encouraging therapeutic results and with lesser side effects noticed during their use, or because they are less aggressive and less harmful for the body[1].The search for new active pharmacological molecules via the screening of natural sources has led to the discovery of a large number of useful drugs that are beginning to play a major role in the treatment of many human diseases [2].

The flora of Algeria is rich of several thousand medicinal species, among this vast natural heritage, our choice was on the *Atriplex halimus* L., commonly called "Guettaf" which is a shrub native of North Africa where it is very abundant it also extends to the Mediterranean coastal areas of Europe and the gypso-saline inland areas of Spain. It is present in regions where the ecological imbalance is accentuated and where the phenomenon of desertification takes alarming dimensions.

It constitutes, during drought and seasonal seals, a forage preferred by Camelidae and particularly sheep and goats. However, overgrazing, climatic constraints and the lack of rational rangeland management have led to a severe degradation of their stands. A species renowned for the nutritional and energetic value of its tender leaves rich in dietary fiber (cellulose), proteins, vitamins (B and C) and mineral salts (sodium,

calcium, potassium, magnesium, phosphorus), not only for livestock, but also as food for nomads and the local steppe population [3].

In addition, it is ranked among the most used plants by the steppe population to treat hyperglycemia [4]. In Algeria it is widely used in therapy mainly for the treatment of different types of cysts "ovarian, uterine and breast cysts".

The aim of our study is to evaluate the antibacterial activity of the methanolic extracts of the aerial parts of the *Atriplex halimus* L. harvested from the region of Biskra (Algeria).

MATERIALS AND METHODS

Plant Material

The species *Atriplex halimus* L. was harvested in the region of Biskra (southeastern Algeria) in December 2018.

The identification of the plant was done with the key to determining the flora of Quezel and Santa [5]. Specimens were kept at the Laboratory of Cryptogamy and Medical Botany, Department of Pharmacy, Faculty of Medicine Annaba-Algeria.

Preparation of Methanolic Extracts

Dry aerial parts (stem and leaf) of *Atriplex halimus* L. have been ground and stored in glass bottles, hermetically sealed at low temperatures. 10 g of the vegetable powder was macerated in 100 ml of methanol with stirring for 24 hours at a temperature of 25 ± 2° C. The extract obtained was filtered and evaporated to dryness under reduced pressure at 50° C on a rotavapor. The dry residue is taken up in 3 ml of methanol and stored at -18 ° C until it is used [6].

The yield of the methanolic extracts was calculated by the following formula: R (%) = (M/M_0) ×100. With: R (%): yield expressed in%; M: mass in grams of the resulting dry extract; M_0: mass in grams of the plant material to be treated.

Phytochemical Screening

Phytochemical tests of the powders of the stem and leaf of *Atriplex halimus* L. obtained are made from precipitation reactions or characteristic staining in order to highlight chemical groups that may be present in this species. The tests are carried out according to the protocols described by Diallo and al., and Senhadji et al. [7, 8]

The results are classified according to the appearance in:

Frankly positive reaction: +++;
Positive reaction : ++ ;
Moderately positive reaction: +;
Shady reaction: ± ;
Negative reaction: -.

Antibacterial test

The test of the sensitivity of the bacteria is carried out by the diffusion method in agar medium (the disk method). It is a method similar to that of the antibiogram which consists in determining the sensitivity of a bacterial strain vis-à-vis one or more substances [6].

The antibacterial activity of the methanolic extracts of the aerial parts (stem and leaf) of *Atriplex halimus* L. is evaluated vis-à-vis sixteen bacterial strains, chosen according to the traditional use of this species in Algeria: *Staphylocoque MRSA, Staphylocoque bucco-dentaire, Staphylocoque ATCC 00, Streptocoque Sp, Entérocoque Sp, Entéroccocus feacalis ATCC 12, Salmonelle, E. Coli BLSE +, Klebsiella pn Marseille, KpC + , Kp ETP R/ IMP R, Serratia Sp, Serratia environnemental, Pseudomonas aerogenosa ATCC 53, Pseudomonas VIM 2*, Bacillus. These strains were kindly provided by the Microbiology Laboratory Manager at Annaba Medical School, Algeria.

Preparation of The Inoculum

By taking sixteen tubes that each contains 5 ml of sterile physiological saline. Using a platinum loop, some well isolated colonies are scraped from each of the bacteria, each of which will be discharged into a tube.

For the preparation of the different concentrations of extracts, 2, 5 mg of each freeze-dried extract (methanolic extract of leaf and stem), are introduced into a labeled tube, in which we added 1 ml of dimethylsulfoxide (DMSO), solvent without any antibacterial effect. The tubes are vortexed until complete dissolution of the extract, and the dilutions are prepared to obtain 1/2, ¼ and 1/8 concentrations from the stock solution.

Seeding should be done within 15 minutes after the preparation of the inoculum. In 16 sterile Petri® dishes, 20 ml of agar are poured. After solidification of the medium, the latter is inoculated with 1 ml of bacteria to be studied. Then, it is spread on the surface using a glass rake.

Sterile 5 mm diameter disks prepared in Whatman® n°1 papers are impregnated with a sterile metal forceps in each concentration and placed on the surface of the solidified medium (Mueller-Hinton) at the rate of 6 disks per box (3 discs of leaf, 3 disks of stem). The dishes were incubated for half an hour at room temperature, then for 24 to 48 hours in an oven at 37 ° C.

The reading is carried out by measuring the diameter of the inhibition zone (Ø), which translates into a translucent halo around each disc; the presence or absence of a halo would explain the sensitivity or the resistance of the germs vis-a-vis extracts tested; according to a symbolic notation scale from - to +++ [9, 10].

Table 1. *Sensitivity of microbial strains according to zones of inhibition*

Sensitivity	Inhibition zone
Not sensitive or resistant (-)	Diameter <10 mm
Sensitive (+)	Diameter between 10 to 16 mm
Very sensitive (++)	Diameter between 16 to 25 mm
Extremely sensitive (+++)	Diameter > 25 mm

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening results characterizing some existing secondary metabolites in the leaves and stems of *Atriplex halimus* L. are shown in Table 2.

The phytochemical screening tests shown in Table 2 show the following results:

-The presence of flavonoids, polyphenols, hydrolyzable tannins, anthocyanin, coumarins, reducing compounds, C-glycosides, cardiotonic glycosides, sterols, carotenoids, iridoids and amino acids in the two parts of the plant studied;
- The absence of alkaloids, saponosides and O-glycosides in the leaf;
- A moderately positive reaction of carotenoids, saponosides in the stem.

Table 2. *Phytochemical Screening Tests*

		Leaf	Stem
Mucilage		++	++
Polyphenols		++	++
Anthocyanins		++	++
Coumarins		++	+
Tannins	Condensed tannins	++	-
	Hydrolyzable tannins	+	++
Flavonoids	Flavonoids	++	++
	Flavonols	++	+
Reducing compounds		++	++
Alkaloids		-	-
Saponosides		-	+ (foam index less than 100)
Anthracene derivatives	Free derivatives	-	-
	O-glycoside	-	-
	C-glycoside	++	++
Cardiotonic glycosides		++	+
Sterols, triterpenes, carotenoids, iridoids	Triterpenes	-	-
	Sterols	++	+
	Carotenoids	+	+
	Iridoids	++	-
	Starch	-	-
	Amino acids	++	+

Extraction Yield

The yield, appearance and color of the methanolic extracts of the stem and leaf of *Atriplex halimus* L. are shown in Table 3.
These results show that:
-The methanolic extract of the leaf is dark green and pasty.
- The yield of the methanolic extract of the stem is the highest, with a percentage of 6.04%, and it is pasty green.

Table 3. *Extraction yield of Atriplex halimus L., by the methanol mixture*

	Extraction Solvent	**Color of the extract**	**Aspect**	**Yield in%**
Stem	Absolute methanol 99%	Green	Pasty	6,04 %
Leaf	Absolute methanol 99%	Dark green	Pasty	5,4 %

Reading Antibiograms

The results of the antibacterial activity are shown in the Table 4.

Table 4. *Inhibition Diameter (mm) of methanolic extracts of Atriplex halimus L.*

	Dilutions of leaf extract			**Dilutions of stem extract**		
	½	**1/4**	**1/8**	**½**	**1/4**	**1/8**
Staphylocoque MRSA	18,2 mm	< 06 mm	27,1 mm	< 06 mm	< 06 mm	<06 mm
	++	-	+++	-	-	-
Staphylocoque bucco-dentaire	< 06 mm	< 06 mm	7,1 mm	7,1 mm	07 mm	07 mm
	-	-	-	-	-	-
Staphylocoque ATCC 00	10,2 mm	11,2 mm	15,2 mm	07 mm	07 mm	07 mm
	+	+	+	-	-	-
Streptocoque Sp	7,1 mm	07 mm	< 06 mm	7,2 mm	8,2 mm	07 mm
	-	-	-	-	-	-
Entérocoque Sp	07 mm	< 06 mm	14,3 mm	< 06 mm	12,1 mm	14,1 mm
	-	-	+	-	+	+
Entéroccocus feacalis ATCC 12	07 mm	11,2 mm	07 mm	14,1 mm	12,1 mm	07 mm
	-	+	-	+	+	-
Salmonelle	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-
E. Coli BLSE +	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-
Klebsiella pn Marseille	07 mm	07 mm	07 mm	9,1 mm	7,1 mm	7,1 mm
	-	-	-	-	-	-
KpC +	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-
Kp ETP R/ IMP R	7,2 mm	07 mm	07 mm	7,2 mm	07 mm	07 mm
	-	-	-	-	-	-
Serratia Sp	07 mm	10,1 mm	12,2 mm	07 mm	07 mm	07 mm
	-	+	+	-	-	-
Serratia environnemental	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-
Pseudomonas aerogenosa ATCC 53	8,2 mm	6,6 mm	8,4 mm	8,3 mm	07 mm	07 mm
	-	-	-	-	-	-
Pseudomonas VIM 2	7,1 mm	7,1 mm	07 mm	07 mm	7,1 mm	7,1 mm
	-	-	-	-	-	-
Bacillus	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm	< 06 mm
	-	-	-	-	-	-

The extraction yields and the characteristics of the extracts depend on the type of solvent used for the extraction and the species. The methanolic extracts of the leaf and stem of *Atriplex halimus* L. are pasty and green or dark green, However, it is difficult to compare these results with those of the bibliography, because the extraction yield is only relative and seems to be related to the extraction methods applied [11], the genetic properties of the species studied [12], at the geographical origin [13] and the conditions for harvesting plant material [14].

The strain sensitivity test showed the presence of some antibacterial activity. According to Table 4, the most sensitive germ is the *Staphylococcus MRSA* with diameters of 27,1 and 18,2 mm for the dilutions of the leaf extract 1/8 and ½ respectively; followed by *Staphylococcus ATCC00* with diameters ranging from 10,2 to 15,2 mm for the range of dilutions of leaf extract ½ to 1/8. For *Enterococcus sp*. and *Enterococcus faecalis ATCC12*, antibacterial activity was observed for dilutions of the stem extract. In addition, leaf extracts and the stem of the *Atriplex halimus* have no activity against gram-negative germs except *Serratia* sp. where we noticed some activity.

Recalling that *Atriplex halimus* contains flavonoids, saponins, tannins, triterpenoids and other phenolic compounds, these compounds having known antibacterial properties, their presence could therefore explain the observed microbial properties.

The difference in the structure of the bacterial wall plays an important role in the susceptibility of bacteria [15, 16]. According to several authors, Gram-bacteria have an outer membrane made of lipopolysaccharides (LPS) which limits the diffusion of hydrophobic compounds [17, 16, 18]. In addition, the periplasm contains enzymes capable of destroying foreign molecules introduced from outside [19], which makes these bacteria generally less sensitive to plant extracts than Gram + bacteria [15, 20, 21]. Moreover, the two methanolic extracts of *Atriplex halimus* L. seem to be more active against Gram + than Gram-. The lipophilic ends of lipoteichoic acids in the wall of Gram + bacteria facilitate the penetration of hydrophobic compounds [22], such as tannins that can reach the cytoplasmic membrane, and disrupt the motive force of proton, active transport and coagulation of cellular contents [16].

The literature reports that the antibacterial activity may be related to tannins which are active compounds of several medicinal plants. They form irreversible complexes with proline-rich proteins, which would result in the inhibition of cell wall protein synthesis. This property has been able to explain the mechanisms of action of plant extracts [23]. They are also effective inhibitors of many hydrolytic enzymes such as the pectolytic enzymes used by phytopathogens [24].

Min et al. (2008) [25] suggest that the source of tannins influences antimicrobial activity. Tannins and gallic acid showed in vitro activity against *B. subtilis, S. aureus and E. coli. K. pneumoniae, L. monocytogenes and H. pylori.* Phenolic compounds, particularly ellagitannins, are strong inhibitory compounds of the genus Staphylococcus. It has been reported that new gallotannins and ellagitannins isolated from the *Punica granatum* fruit crust are the main components responsible for the antimicrobial action [26]. It has been suggested that gallotannins of *Galla chinensis* are effective antibacterial agents [16].

The glycosylated flavonols isolated from the aerial parts of *Brunfelsia grandiflora* and some of these structures are known to be responsible for the antibacterial activity [27]. This confirms the work of Rojas et al., 1992 and Marjorie, 1999, [28, 29], which showed that flavonoids, triterpenoids and tannins, as well as other phenolic compounds

or free hydroxyl groups, are classified as very active antibiotic compounds. In addition, it has been shown that the mechanism of toxicity of flavonoids to microorganisms is either by deprivation of metal ions such as iron, or by non-specific interactions such as the establishment of hydrogen bridges with the cell wall proteins of microorganisms (adhesins) or enzymes [12].

CONCLUSION

The diffusion method on agar medium made it possible to demonstrate the antibacterial power of the methanolic extracts of the leaf and the stem of the *Atriplex halimus* L. from Biskra, Algeria, vis-à-vis the sixteen bacterial strains tested. The leaf extract showed antibacterial activity against Gram-positive bacteria as Gram-negative bacteria.

Following these results, it would be interesting to extend the range of antimicrobial tests to other microbial agents to confirm their effectiveness. As it is essential to look for new effective antibacterial substances with broad spectrum of action. All these results obtained is only a first step in the search for biologically active substances of natural origin. A chemical analysis is desirable to obtain a more in-depth view of the qualitative and quantitative composition of these extracts studied in order to highlight the therapeutic effect of this medicinal species.

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Research Article

DETERMINATION OF PHENOLOGICAL CHARACTERISTICS OF SOME LOCAL GRAPEVINE (*Vitis vinifera* subsp. *sativa*) GENOTYPES COLLECTED FROM ERKILET REGION IN KAYSERI

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ABSTRACT. This study was conducted to determine the phenological characteristics of some local genotypes in the vineyard areas from Erkilet region of Kayseri province in 2018. A total of 11 different local grape genotypes known, loved and widely used by the local people for many years in cultivation were chosen. During the vegetation period, bud burst and full bloom observations were made and recorded as day/month. As a result of the study, differences were found among local genotypes in terms of phenological stages. The earliest budburst was recorded on 20 March and the latest budburst was observed on March 29. The full bloom time has ranged from May 20 to May 30 among genotypes.

Keywords: *budburst, bloom, grapevine, Kayseri, local genotypes*

INTRODUCTION

Turkey is the most favorable climate in the World for viticulture and has an old and long history culture [1]. Turkey has a very rich variety of species and types in terms of viticulture, and offers very important gene resources that can be used in breeding studies. Especially, local varieties have a great importance in the development of new varieties [2].

Characterization and evaluation of such invaluable genetic resources is the necessity to avoid genetic erosion. Ampelography is based on the morphological and phenological and pomological characteristics of vine species and varieties. It is important for collection, protection and accuracy of gene resources.

Kayseri; is a city with a long history of vineyards, famous for its vineyards and grapes. In addition to being an important grape production center, it has a very rich genotype variety in terms of viticulture.

Erkilet region in Kayseri provinces has a vineyard area since an ancient time and has a significant diversity. In terms of many varieties and types of richness in viticulture, can be used in breeding programs offer very important gene resources.

This study was conducted to determine phenological characteristics of some local genotypes in the vineyard areas from Erkilet region of Kayseri province.

MATERIALS AND METHODS

Material

This study was carried out in the local producer vineyard areas determined and representing in Erkilet region of Kayseri province in 2018 (Fig. 1 and Fig. 2).

***Fig. 1**. Kayseri province in Turkey*

***Fig. 2.** Erkilet vineyards in Kayseri*

A total of 11 different local grape genotypes which is known and widely used by the local people for many years used in this study (Fig. 3).

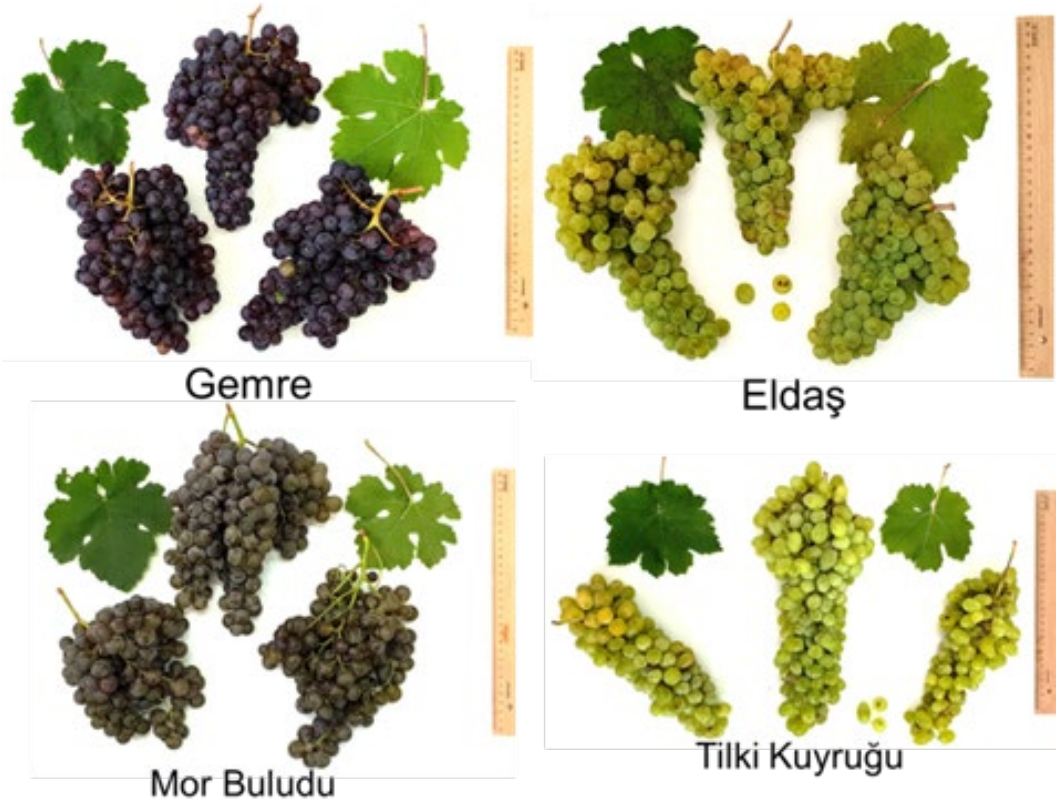

Fig. 3. *Some local genotypes in Erkilet region*

Method

During the vegetation period, bud burst and full bloom observations were recorded as day/month according to the Descriptors for Grapevine (*Vitis* spp.) [3]. (Fig. 4).

Fig. 4. *Budburst and full bloom observations in Erkilet region*

RESULTS AND DISCUSSION

According to results, some differences were found among local genotypes in terms of phenological stages (Table 1). The earliest budburst was recorded on 20 March with Mor buludu named ERK 01 and the latest budburst was observed on 29 March Sungurlu (ERK 10) and Göğcek (ERK 11).

The full bloom time has ranged from 20 May and 30 May among the genotypes. The earliest flowering genotypes were Mor buludu, Gemre, and Misket.

A difference of about 10 days was found between genotypes in terms of the bud burst and full bloom dates depending on the direction and elevation of the vineyard.

Table 1. *Phenological stages of local genotypes*

Genotype no	Local name	Budburst	Full bloom
ERK 01	Mor buludu	20.Mar	20.May
ERK 02	Gemre	23.Mar	20.May
ERK 03	Misket	21.Mar	20.May
ERK 04	Beyaz buludu	22.Mar	30.May
ERK 05	Mor buludu	28.Mar	30.May
ERK 06	Tilki kuyruğu	28.Mar	30.May
ERK 07	Şireder	28.Mar	30.May
ERK 08	Parmak üzüm	28.Mar	30.May
ERK 09	Eldaş	27.Mar	28.May
ERK 10	Sungurlu	29.Mar	26.May
ERK 11	Göğcek	29.Mar	26.May

Phenological development stages differed in the same ecology but in different vegetation years due to changes in climate data [4]. Budburst in wine grape varieties grown in Diyarbakır was between 10-20 April and full bloom between 1-6 June [5]. In another study, bud burst of some grape cultivars were found as; 12-24 April and full bloom dates between 4-11 June [6].

Different results were obtained from previous studies carried out in different regions regarding phenological characteristics. Budburst times of cultivars were 4-20 April [7], 15-27 April [8].

As a result, genetic factors and ecological conditions may be the cause of the different results in the studies performed in different regions.

CONCLUSION

In this study, it has been determined that phenological development periods in vines vary depending on the cultivars, years and ecology. According to the meteorological data, the Spring of 2018 appears as the hottest spring season seen in the records [9]. In our study, it was determined that earliness was provided in the Erkilet region in terms of budburst and full bloom dates.

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Research Article

MICROPROPAGATION OF DIFFERENT PITAYA VARIETIES

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ABSTRACT. Pitaya attracts attention due to its properties such as fruit color, minerals, antioxidant properties and rich nutrient content both in Turkey and in the world. Seed and cuttings are mostly used in the production of pitaya. One of the best methods for fast and disease-free production of pitaya is *in vitro* tissue culture. In this study, young shoots of different pitaya varieties were cultured in Murashige and Skoog (MS) basal medium supplemented with different plant growth regulators such as 6-benzylaminopurine (BAP), gibberellic acid (GA_3), Indole-3-butyric (IBA). The highest value of the multiplication coefficient (5.41) was found in Halley's Comet variety cultivated in MS medium supplemented with 2.0 mg/l BAP. The lowest value (1.84) was detected in Bloody Mary cultivar growing in MS medium supplemented with 2.0 mg/l BAP. According to the results of rooting studies, between 10-95% root formation was detected when the medium and pitaya varieties were compared. While the best medium for rooting, MS medium supplemented with 1 mg / l IBA is detected, It has been determined that MS medium without plant growth regulators (PGRs) can also be used for rooting. Based on our results, the healthy and large amount of pitaya seedlings could be obtained.

Keywords: *Pitaya, micropropagation, American Beauty, Halley's Comet, Vietnam White and Bloody Mary*

INTRODUCTION

Pitaya or dragon fruit, which has attracted attention due to its attractive appearance, taste, and nutritional content in recent years, is located in the genus *Hylocereus* of the Cactaceae family of the Caryophyllales order. It is known by many names in the world [1, 2, 3]. Some of those are pitahaya, night-blooming cereus, strawberry pear, dragon fruit (in Southeast Asia), and päniniokapunahou or päpipi pua (in Hawaii) [4]. Its homeland is Central America and Mexico. Nowadays, it is commercially produced in the Bahamas, Bermuda, Indonesia, Colombia, Israel, Philippines, Maymar, Malaysia, Mexico, Nicaragua, North Australia, Okinawa (Japan), Sri Lanka, South China, South Florida, Taiwan, Thailand, Vietnam, El Salvador, Venezuela, Colombia and West Indies [5, 6, 7].

Pitaya varieties consist of 3 main types: *H. polyrhizus*, *H. undatus*, *H. guatemalensis* and interspecific hybrids [8, 9]. One of the other important species is reported to be *H. megalanthus*. Different *Hylocereus* species can be distinguished according to the shape, color, and size of the fruit pulp. The most commercially grown species are *H. polyrhizus*, *H. megalanthus* and *H. undatus* [10, 11].

All dragon fruit varieties have flesh filled with a large number of edible black seeds. Its shoots are green and fleshy and can be three, four and five-pointed. There are aerial roots that hold on to trees and rocks. Apart from clinging, these roots collect nutrients and nutrients in the medium, helping the plant to nourish. Plants can be reproduced by cutting from the places where these roots are. Although fruit weights reach up to 900 gr, it is on average between 350 and 450 gr. Although Pitaya is mostly used as a fruit, it is also used

as health products and medicine. It has delicious fruits and can be used for making juice, marmalade, vinegar, jam, ice cream and wine. Its fruits are essential for health and contain vitamin C, phosphorus, iron, vitamin B1, vitamin B2, vitamin B3 and many other compounds. Ascorbic acid value per 100 g fresh dragon fruit has been reported as 25 mg for *H. undatus* [1, 7, 8]. A nitrogen-containing compound known as betalains or especially red betacyanin is effective in red color formation in fruit and peel [12, 13]. Betacyanins are used commercially as natural food dyes [3].

Fruit seed oils are a good source of essential fatty acids and tocopherol with high stability [14]. It has been reported that some pitaya seeds contain essential fatty acids, especially linoleic acid, and linolenic acids for human health. When the % composition of fatty acids of *H. polyrhizus*, *H. undatus* and *H. meglanthus* are evaluated separately, it was reported as 23.5%, 21.0%, 18.8% for total SFA (saturated fatty acids) respectively and 26.3%, 23.9%, 14.3% for MUFA (monounsaturated fatty acids) respectively and 48.7%, 53.8%, 65.4% for PUFA (polyunsaturated fatty acids) respectively. The highest linoleic acid was reported in *H. meglanthus* with 65.4% and the highest oleic acid was reported in *H. polyrhizus* with 25.5% [3, 15, 16].

The production of Pitaya is traditionally performed through cuttings. Productions by cuttings are insufficient for commercial productions due to the lack of young nodes. Besides, Pitaya is also produced with seed but shows genetic expansion in seed production. Rapid, quality, and high-efficient micropropagation methods of some pitaya species is a very significant step for commercial production. Most researchers have focused on the cultivation technology of the plant [17, 18, 19, 20, 21, 22], extraction of components [23, 24, 25] and tissue culture methods [26, 27, 28, 29, 30, 31, 32, 33]. It can show different effects on pitaya varieties of medium supplemented with different plant growth regulators (PGRs) used in tissue culture studies. Researchers argued that this was due to the different genetic background and genetic condition that the pitaya has [34, 35, 36].

In this study, it is aimed to create a reliable and fast method for commercial production of different pitaya varieties that may be of commercial importance. *In vitro* proliferation and rooting of different pitaya varieties were evaluated using the plant tissue culture method, which is one of the important methods of plant biology, using MS basal medium [37] supported by combinations of different PGRs.

MATERIALS AND METHODS

This research was carried out in the Biotechnology Laboratory owned by Tekfen Agricultural Research Production and Marketing Inc. Adana, Turkey.

Plant material and Source of Explants

Plant materials were obtained from growers in Mersin, Turkey. Young shoot tips of pitaya varieties known as American Beauty, Halley's Comet, Vietnam White and Bloody Mary were used a source of explants for *in vitro* tissue culture.

Media and Culture Conditions

Initial plant materials were firstly sterilized by surface sterilization. Explants were subsequently kept under running tap water for 20 min and washed with dishware detergent and then rinsed by pure water. Then, explants were kept in 70% ethanol for 20

seconds and then 20 minutes in 1.5% (v/v) sodium hypochlorite. Finally, the explants were washed 3-4 times with distilled water.

MS medium supplemented with 2,0 mg/l BAP (M1) and 4.0 mg/l BAP (M2) was used for micropropagation of plants. For the rooting of the explants, MS medium supplemented with 1mg/l IBA (R2), 0.5mg/l GA_3 (R3), 0.5mg/l IBA + 0.5mg/l IBA (R4) and hormone-free MS (R1) medium used. Agar (7,5 g/l) was added to solidify the medium and the media pH was adjusted to 5.8 using 1 N HCl and 1 N NaOH. The prepared media were autoclaved at 121℃ at 15 psi for 20 min. and distributed in disposable plastic containers.

Explants with completed surface sterilization were cultured on MS basal media supplemented with different PGRs and aseptic plants were obtained. After the explants were transferred to the medium, they were transferred to plant growth chambers. All cultures were incubated under 36 W cool white fluorescent lights in a light / dark photoperiod for 16/8 hours and at 25 ± 2°C. Micropropagation trials continued by being subcultured every four weeks in a fresh medium. Micropropagation was evaluated at the end of 3 subcultures and the rooting studies at the end of 6 weeks.

Statistical Analysis

All trials were set up with three replications according to completely randomized design. All quantitative data calculated as percentage value were subjected to arcsine transformation before variance analysis. The data were analyzed using JMP-8 (SAS Institute Inc., NC, USA) statistical package program and variance analysis was performed. The differences between them were compared with the LSD (least significant difference) multiple comparison test.

In micropropagation trials, the multiplication coefficients were determined by considering the number of shoots per explant. In this context, both varieties and micropropagation mediums were evaluated. In rooting studies, root length (cm), % rooting, shoot length (excluding root) (cm) were evaluated (Figure 1).

RESULTS AND DISCUSSION

Contamination was not observed in any of the cultures and successful results were observed. Production of plants by *in vitro* tissue culture method has been investigated. *In vitro* micropropagation and rooting of different pitaya varieties on MS medium supplemented with different combinations of PGRs were evaluated. As a result of our studies, different types of pitaya responded differently to different mediums. For this reason, it is especially important to develop protocols for each type and this research has the data to provide this.

In this study, the highest shoot average per explant was obtained from M2 coded medium containing 4.0 mg/l BAP. The average multiplication coefficient was found to be 3.34 statistically. The highest shoot development average per explant was observed in Halley's Comet variety with 5,27. The highest multiplication coefficient was determined with M1 medium and Halley's Comet type with 5.41. The lowest multiplication coefficient was found in M1 medium and Vietnam White variety with 1.84 (Table 1). Similarly, Khalafalla et al. [38] stated that MS medium supplemented with plant growth regulators [(benzyladenine (BA), kinetin (Kin), naphthalene acetic acid (NAA)] has a positive effect on the shoot development of *Opuntia ficus-indica*. They found the highest shoot multiplication in MS medium supplemented with 5.0 mg/l BA. Researching with

different PGRs, Mohamed-Yasseen [27] observed the best shoot proliferation of *H. undatus* in medium containing 0.5mM TDZ and 0.5mM NAA (naphthaleneacetic acid).

Table 1. *The effect of different media on the efficiency of multiplication of in vitro propagated different pitaya varieties*

	Medium	Multiplication coefficient
American Beauty	M1	2.38d
	M2	4.12c
Halley's Comet	M1	5.41a
	M2	5.14b
Bloody Mary	M1	1.26g
	M2	2.12e
Vietnam White	M1	1.84f
	M2	2.00ef

*LSDvariety: 0,122***, LSDmedium: 0,086***, LSDvariety x medium: 0,173****

Suárez Román et al. [39] conducted studies on the reproduction of Yellow (*Selenicereus megalanthus*) and Red (*H. polyrhizus*) pitaya by *in vitro* tissue culture. It has been observed that the mean of the multiplication coefficient of the red pitaya (3.2) is higher than the yellow pitaya (3.0). In our study, this value was found between 1.69 and 5.27. When the researchers evaluated the medium, the highest mean multiplication coefficient was found in MS medium containing 2mg / l BAP + 2mg / l Kinetin and the lowest multiplication coefficient was found in MS medium with (1.4) 5mg / l BA + 0.05mg / l NAA (naphthaleneacetic acid). In our study, it was found 3.34 in M2 medium and 2.72 in M1 medium.

In root trials, as in micropropagation, Halley's Comet was the best variety according to Shoot Length with an average of 3.17 cm. When the medium and the pitaya variety are evaluated together, the highest value (4.02 cm) was determined in the Halley's Comet variety developing in the R2 medium. In general, shoot length was determined to be between 3.17-1.07 cm in plant varieties (Fig. 1) (Table 2).

Fig. 1. *Root length and shoot length measurement of Halley' s Comet rooted in R2 medium under in vitro culture conditions*

Considering the root lengths, Bloody Mary (3.18 cm) is the type that gives the best results according to the average root length. Although there was no statistically significant difference between R1 and R2 mediums, it was determined that the two medium were successful in terms of root length. Accordingly, it was observed that the hormone-free MS medium might be sufficient for the lengthening of the roots. Similarly, Clayton et al. [40] found that 11 rare or endangered cactus species provide successful rooting in hormone-free MS medium.

Table 2. *Effect of different mediums on root formation, shoot length and root length (Values in parentheses are angular transformation values of percentage of response)*

	Medium	Shoot Length (cm)	Root length (cm)	Rooting percentage (%)
American Beauty	R1	2.34 d	3.48 d	35 fg (36,23)
	R2	2.85 c	2.16 f	90 ab (71,95)
	R3	1.60 ef	1.20 g	25 hı (29,92)
	R4	1.45 f	1.09 g	55 c (47,87)
Halley's Comet	R1	3.36 b	5.51 b	50 cd (45,00)
	R2	4.02 a	3.80 d	95 a (76,89)
	R3	3.60 b	1.20 g	30 gh (32,96)
	R4	1.70 ef	1.04 g	30 fgh (33,59)
Bloody Mary	R1	1.73 e	5.35 b	20 ı (26,88)
	R2	1.62 ef	6.11 a	90 b (70,78)
	R3	0.44 h	0.10 ı	10 j (20,40)
	R4	0.90 g	1.18 g	40 ef (38,63)
Vietnam White	R1	1.11 g	2.60 e	45 de (41,73)
	R2	1.56 ef	4.55 c	90 b (71,33)
	R3	1.07 g	0.55 h	10 j (19,21)
	R4	0.56 h	0.91 gh	25 hı (30,20)

*Shoot Length: LSD variety: 0.126***, LSD medium: 0.126***, LSD variety x medium: 0.253***, Root length LSD variety: 0.183***, LSD medium: 0.183***, LSD variety x medium: 0.367, Rooting percentage: LSD variety: 2.655***, LSD medium: 2.655***, LSD variety x medium: 5.311****

El Finti et al. [41] studied *in vitro* micropropagation of *Opuntia ficus-indica* and observed rooting both in media containing PGRs and in media without PGRs. They detected 100% rooting in all mediums. They observed the best root formation in medium containing indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA). In our study, rooting success varied considerably (10-95%). This variability is thought to be due to both different pitaya varieties and the PGRs used. Emphasizing that plants need essential nutrients in addition to phytohormones in their growth and development, Zhe Cheng et al. [42] examined the effects of waste coconut water and sucrose on micropropagation of *H. polyrhizus*. They used MS medium containing 0.03 mg/l BAP and 0.01 mg/l NAA supplemented with different concentrations (0%, 2%, 4% and 6%) of waste ripe coconut water and sucrose (0%, 1%, 2% and 3%). They found that waste coconut water had a positive effect on the elongation of the shoots while it did not have any effect on root

induction. In our study, different nutrients or elicitors were not used. However, successful results were obtained in both shoot elongation and rooting. In a different study, Sheng et al. [43] examined the effects of plant growth regulators on germination of pitaya seeds in vitro conditions. In their study, they investigated the germination percentage of seeds in semi-solid MS medium supplemented with 1 ppm BAP and 3 doses of IBA (0.0-0.50.8 ppm). They reported that the application with the highest germination rate (93.33%) was a combination of 1 ppm BAP and 0 ppm IBA. They obtained the highest callus induction percentage (75%) for *H. costaricensis* from the combination of 3.6 ppm 2.4D and 1.8 ppm BAP. In our study, there was no study with pitaya seeds since genetic variability may occur. Callus formations were constantly observed in in vitro cultures but were not taken into consideration. It is clear that callus culture studies in pitaya tissues can be used to produce secondary metabolites. Our study to meet commercial production demand and establish health micropropagation protocols based on varieties will guide other researches.

CONCLUSION

This study provides a protocol for micropropagation and rooting of different pitaya varieties. The effects of the media prepared with different PGRs on the micropropagation of different pitaya varieties were evaluated. Media and pitaya varieties were analyzed in terms of growth factor, root formation, root length, shoot length. When the multiplication coefficients were examined, the highest value (5.41) was determined in Halley's Comet cultivar grown in MS medium supplemented with 2.0 mg/l BAP. Halley's Comet has been observed to be successful in root formation as in micropropagation. M2 coded medium containing 4.0 mg/l BAP was determined as the best medium according to the multiplication coefficient averages. R2 medium containing 1 mg / l IBA was observed to be successful in rooting. Vietnam White has been found to be more unsuccessful in both micropropagation and rooting than other pitaya varieties. It is predicted that successful results will be obtained from different studies. The results obtained will provide highly efficient reference protocols for micropropagation of pitaya.

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Review Article

NEW APPROACHES TO PROTECT WILD ALMOND GENETIC SOURCES: A REVIEW

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ABSTRACT. Turkey has rich local and wild fruit varieties which grown at poor soils and hard climatic conditions. One of them is wild almonds which spreads almost everywhere at Anatolia. Cultivated almonds became differentiated into two ecotypes during evolution and later domestication processes: Mediterranean and Central Asia. Numerous related wild species are also found growing in the mountains and deserts of Central Asia from western China through Iran and Turkey. These native almond species are utilized for oil extraction, soil erosion control, reforestation, rootstocks, and as a source of novel genes in breeding programs. But important wild almonds have lost because of road construction and new residential regions in recent years. To prevent this, it needs new strategies such as genetic material collection, genetic characterization and the use of biotechnology to breed new cultivars. In this study, we discussed new approaches to protect wild almond genetic sources.

Keywords: *Wild almond, genetic approaches*

INTRODUCTION

Almond (*Prunus dulcis*), in Turkish, this species is called 'Badem', is a species of genus *Prunus* and subgenus *Amygdalus* that is commercially grown worldwide. Almond is one of the most important nut crops worldwide and it produces fruits of high commercial value. USA is the leading producer with about 1 million tonnes production [1]. In the year 2018, the world production of almond was about 3,2 million tonnes out of which Turkey produced 100,000 tonnes [2]. Almond species grow in regions of the world that are characterized as having a subtropical Mediterranean climate, with mild wet winters and warm, dry summers. These species originated in central Asia and represent divergent evolution under xerophytic environments [3]. Its production is mostly based on orchards with traditional managements. These clones, local cultivars and seedlings as well as related wild species constitute a valuable source of genetic diversity and an excellent potential for improvement. Owing to the responsibility of rootstock for a wide range of fruit tree properties, they have an important role in modern horticulture and commercial orchards [4-7].

The *Prunus* species *P. fenzliana* (Fritsch) Lipsky, *P. bucharica* Korschinsky and *P. kuramica* Korschinsky (of the Section Euamygdalus) from its origin regions are described as the wild species most closely related to almond [8,9], and may be the ancestral species of the modern cultivated almond [1]. There are more than thirty wild or partially cultivated almond species in the world. Ladizinsky [10], however, identified only *P. fenzliana* as the wild ancestor of almond. *P. webbii* (Spach) Vieh, thought to have originated on the Balkan peninsula, is also described as closely related to almond [8,9]. The evolution and distribution of almonds, both in cultivation and in

the associated semi wild state, has been divided into three stages: Asiatic, Mediterranean, and Californian, corresponding to the geographical areas where is grown [8, 1, 11, 12].

The fruit of almond, as with other *Prunus* species, is a drupe where the mature, stony endocarp together with the seed forms a propagation unit comparable to a botanical seed surrounded by its protective testa. The almond is the earliest deciduous fruit and nut tree to bloom in spring due to its low winter chilling requirements and quick growth response to warm temperatures [1,11,12].

Almond Breeding Objectives

The limited gene pool in cultivated almond limits the cultivation of specific areas with Mediterranean climate. Related almond species demonstrate a higher resistance to abiotic and biotic stresses and so represent valuable germplasm sources for breeding [13]. Crosses between almond and related species have been successful [14,8,15,11,16] and numerous spontaneous interspecific hybrids have been reported [17,18,9]. Interspecific hybrids between these related species, including peach [*P. persica* (L.) Batsch] 9 almond and *P. webbii* Spach 9 peach have been previously used long time for almond rootstock breeding in France [14], USA [19], Spain [20] or Yugoslavia [21]. In addition, many of these species have been used directly as a rootstocks for almond usually for non-irrigated conditions, including *P. spartioides* Spach [22] in Iran; *P. bucharica* (Korsh.) Hand.-Mazz. [23,24] and *P. fenzliana* Fritsch [25] in Russia; *P. webbii* [26] in Turkey; and *P. fenzliana, P. bucharica, P. kuramica* Korsh. and *P. argentea* Lam. [8] or *P. dehiscens* Koehne and *P. kotschyi* (Boiss. et Hohen.) Nab. [27] in France.

Why Plant Gene Resources to Be Reduced and Lose Rapidly?

The rapid increase in the world's population, unconsciously utilizing herbal resources for human needs, land trenches, traditional varieties have been replaced with hybrids, use of herbicides, consuming from nature instead of production, natural disasters, urbanization and industrialization cause plant gene resources to be reduced and lost rapidly [28].

Development of new varieties to increase agricultural production and transposing to the wild plant species in the state of raw material to future generations without erosion will be possible with the preservation and protection of the existing plant variety.

Although the gene resources in the world are protected by the gene banks, the reasons for the fact that the gene banks have limited opportunities and the number of gene sources to be protected is high; new techniques for collecting, evaluating and reproducing gene resources are used [29].

New Approaches to Protect Wild Almond Genetic Sources

Biochemical and Molecular Techniques

In order to supplement the morphology-based results, several molecular techniques, including isoenzyme [30] and DNA-based markers such as ISSRs [31,32], SSRs [33,12,34-38] and AFLPs [39,40] have been used for describing diversity and genetic characterization of wild type almond germplasm throughout the world. These techniques are not affected by environmental changes and have a short time like a day.

In Vitro Techniques

Tissue culture techniques used for vegetative replication are used to store problematic gene sources for many years, but embriyonic suspension techniques ensure that the protecting is at the cell level.

The preservation of genetic material in the form of DNA has provided new possibilities for the conservation of plant genetic resources [41-44].

Slow Growth Technique Using Enclosure

The main of this technique is to minimize the growth rate of cultures *in vitro* storage. For this purpose, successful results can be obtained by

•using immature zygotic embryos,
•reducing storage temperature,
•reducing oxygen pressure in the culture media,
•modification the media composition,
•pouring leaves of shoots.

Cryopreservation

The principle of this method, which is developed for long-term storage, is that the genetic material is frozen at very low temperatures [29].

The cultures are frozen with liquid (-196 °C) or gas (-150 °C) nitrogen. At the end of storage, the temperature is increased and cell proliferation studies can be carried out when the material is ready. [45,46].

Artificial Seed Storage

This method is mostly used for preserving the species that are resistant to drying and storing seeds. In this method, shoot tips or somatic embryos are encapsulated with the help of a half-fold material that serves as seed coats and endosperm (Fig. 1) [29, 47].

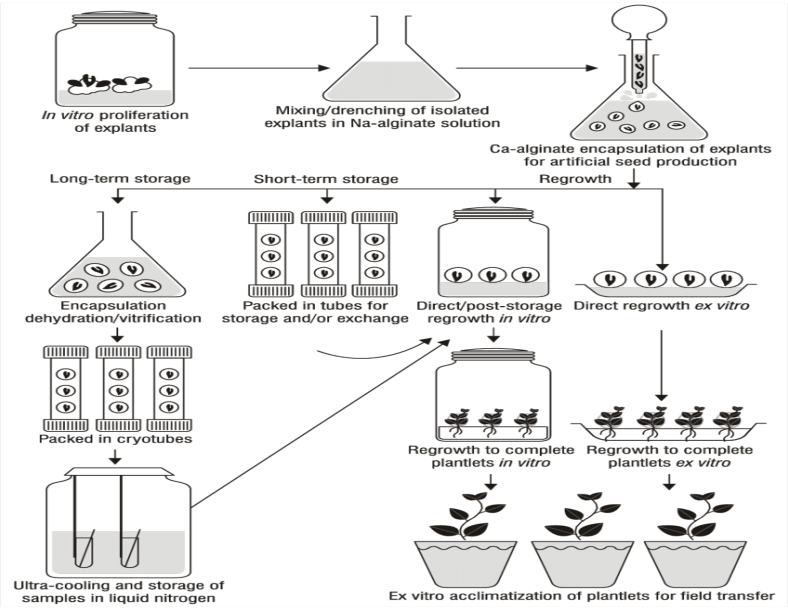

Fig. 1. *Artificial seed storage*

CONCLUSION

These wild species provide an enlarged pool of available germplasm and suitable characteristics such as late bloom and self-fertility and resistance to drought, salinity and low winter temperatures. Moreover, wild almond species demonstrate higher resistance to abiotic and biotic stresses, and so represent valuable germplasm sources for breeding.

These native almond species are utilized for; oil extraction, soil erosion control, reforestation, rootstocks and as a source of novel genes in breeding programs. But important wild almonds have lost because of road construction and new residential regions in recent years. To prevent this, it needs new strategies such as genetic material collection, genetic characterization and the use of biotechnology to breed new cultivars.

In this study, we discussed wild almond cultivars and new approaches to protect these genetic sources.

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