established in 2016

MAS JOURNAL of Applied Sciences

ISSN 2757-5675

DOI: http://dx.doi.org/10.52520/masjaps.182

Araştırma Makalesi

In vitro mass propagation of *Muscari neglectum* Guss. Ex. Ten.

Çiğdem Alev ÖZEL[1]*, Fatma ÜNAL[2]

[1]Gazi University, Department of Biology Education, Faculty of Gazi Education, Teknikokullar, Ankara

[2]Gazi University, Department of Biology, Faculty of Science, Teknikokullar,Ankara

*Corresponding author: cigdemozel@gazi.edu.tr

Geliş Tarihi: 20.09.2021 **Kabul Tarihi**: 25.10.2021

Abstract

Muscari neglectum Guss Ex Ten, in the Asparagaceae family, is a geophyte with blue flowers, distributed in Mediterranean countries including Turkey, South East England, Central Russia, Caucasus and, Iran. It is a species that is gradually decreasing in its natural habitats due to increased urbanization and many other anthropological factors. Therefore, rapid reproduction of the plant with tissue culture studies for conservation is of particular importance. For this purpose, *M. neglectum* bulbs were disinfected in 80% commercial bleach for 20 minutes. This was followed by culturing double scales, and the bulbs obtained *in vitro* to regenerate on a medium containing various concentrations of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA). The highest bulb regeneration (8 bulblets) from double scale explants was observed on MS basal medium containing 4 mg L^{-1} BAP+0.5 mg L^{-1} NAA. A maximum primer bulblet diameter of 0.48 cm was noted on MS basal medium containing 2 mg L^{-1} BAP+2 mg L^{-1} NAA using 2 cm bulbs. The number of secondary bulbs ranged from 1.00-3.64. All of these bulbs are rooted in MS basal medium. The highest rooting (7.83 roots/bulb) was observed on the bulbs taken from the medium containing 2 mg L^{-1} BAP-2 mg L^{-1} NAA. The longest root formation was obtained from the bulbs with 14.2 cm on 2 mg L^{-1} BAP-0.5 mg L^{-1} NAA. Bulbs were transferred to pots and their adaptation to the soil was ensured.

Keywords: Disinfection, regeneration, doubles scales, 2 cm bulbs, rooting, adaptation

INTRODUCTION

There are more than 50 known species of the genus Muscari in the Mediterranean region, covering Europe, North Africa, and Turkey (Chittenden, 1956; Van Scheepen, 1991; Jafari and Maassoumi, 2011; Govaerts, 2019; Yıldırım, 2020). All of these species have a crucial role in the rich biodiversity of Turkey. The Muscari genus has been revised several times by many researchers. After the last revision, the checklist (Eker, 2012) has counted 49 species in Turkey (Yildirimli, 2010; Eker, 2012; Eker, et al., 2019; Eker, 2019a, b; Kayiran et al., 2019; Eker, et al., 2020a, b; URL-1; URL-2; URL-3). As per the latest two revisions, the genus Muscari in Liliaceae family has been transferred to Hyacinthaceae family and then replaced in Asparagaceae family (Eroğlu, 2020). While the majority of the Muscari species have been described between the Mediterranean and the Irano-Turanian phytogeographical regions, having high endemism for plant diversity, populations of this geophyte are gradually decreasing in their natural habitats due to uncontrolled exports in the past, unplanned rapid urbanization, and many other anthropological factors. The genus Muscari is included in the group of taxa that exist at the level of medium-term in nature and are highly threatened in the future (Ekim et al., 2000). Therefore, the export of flower bulbs belonging to any species in the genus Muscari is banned and prohibited for export in legal terms by the Turkish Republic Ministry of Agriculture and Rural Affairs in a notification that was published the first time in 1989 and is constantly revised and updated every year (URL-4). *Muscari neglectum* Gus. Ex Ten is an important species in the genus Muscari. It is a bulbous monocot plant with flowers (Lim 2014). It spreads on Pinus groves, scrubs bushes, meadows, calcareous rocky slopes, and dunes. *M. neglectum* blooms in spring. This plant is found in Turkey at (1a) Strandja Section, (1b) Çatalca-Kocaeli Section, (1ç) Southern Marmara Section, (2a) Western Black Sea Section, (2b) Central Black Sea Section, (3a) Main Aegean Section, (3b) and Central Western Anatolian Section, (4a) Upper Sakarya Section, (4c) Upper Kızılırmak Section, (5a) Upper Euphrates Section, (6a) Antalya Section, and (6b) Adana Section (Güner et al., 2012). It spreads up to 2,300 m above sea level (URL-1). Its local name is "Arap üzümü" (Arabian grape) (Eker, 2012). Muscari species are crucial geophytes with commercial importance in the ornamental and medicinal plants sector (Yücel Şengün and Öztürk, 2018; Meydan, 2019). Lim (2014) has indicated that it is served in Mediterranean dishes or eaten as salad and brined pickle with numerous nutritional and medicinal properties. Many antimicrobial activities and medicinal properties have also been reported in *M. neglectum* (Goktas et al. 2010; Nasrabadi et al., 2013; Eroğlu-Özkan et al., 2017; Semerci et al., 2019). Rohollahi and Naji, (2020) has conducted molecular studies to determine its level of genetic diversity.

After germination of *M. negluctum* seeds, they take a long time such as 3-5 years to bloom and set seeds; therefore, the development of new, easy and faster methods for the agronomic and *in vitro* production of this geophyte are desired and are of particular importance (Ozel, 2008; Kocak et al., 2019). Studies on *in vitro* development of *M. neglectum* have been performed by several investigators (Karamian and Ranjbar 2010; Karamian, et al., 2011; He et al. 2016; Ozel and Unal 2016). Rapid reproduction of the plant with tissue culture studies for conservation and production is of particular importance. However, there is

still a need for efficient regeneration of this plant using various protocols. Therefore we aimed to develop an effective and successful application. For this purpose, *M. neglectum* bulb scales and *in vitro* grown bulbs were regenerated on MS basal medium having several combinations or concentrations of 6-Benzylaminopurine (BAP)+ 1-Naphthaleneacetic acid (NAA). The procedures developed would help to facilitate the regeneration potential of *M. neglectum*.

MATERIAL and METHOD

Collection and identification of the experimental materials

M. neglectum bulbs were collected from the natural growing area of Ankara province and diagnozed by Prof. Dr. Seher Karaman Erkul.

Culture conditions and explant surface disinfection of explants

The bulbs were stored in a cool (15±1°C), dry (40% humidity), and dark chamber for 28 days. Subsequently, they were disinfected in 4% commercial bleach (having 5% NaOCl -Ace - Turkey) for 20 min. Tween 20 was used as surfactant at the rate of 1% (v/v). The disinfected bulbs were rinsed for 5 × 3 min using bidistilled sterilized water. Subsequently, these bulbs were vertically sliced into 4 pieces ensued by taking two bulb scales each attached at the bottom with a thin basal plate binding section. All disinfected explants were also subjected to Plant Preservation Mixture (PPM) (1%; v/v) for 60 min. The disinfected twin bulb scales were placed on MS basal medium (Murashige and Skoog, 1962) having 7.0 g L^{-1} Agar Type A (Merk, Germany) and 30 g L^{-1} sucrose (Merk, Germany) for 48 hours to disinfect them. These were previously autoclaved at 121°C and 106 kPa for 20 min. The pH of all the cultures was maintained at 5.6 - 5.8. The selected healthy twin bulb scale explants were treated with 1.0, 2.0, and 4.0 mg L^{-1} BAP, and 0.5, 1.0, and 2.0 mg L^{-1} NAA in MS basal medium (total 9 combinations). Afterward, the explants were regenerated under 16 h daylight (35 µmol photons m^2 s^{-1}) at 24 ± 1°C using Philips daylight lamps (TLD 36 W/54, Hungary) photoperiod for 56 days. Sticking agar on their roots was carefully removed using warm water. The rooted bulblets were transferred to pots filled with compost. A micro-climate was created by enveloping the potted bulbs with transparent 110 gauge polyethylene sheets for maintaining 80% relative humidity at 24±1°C for 12 days. Thereafter, the enveloping sheets were holed for air exchange and relative humidity was gradually reduced to 40% without disturbing the acclimatization process. The polythene bags were completely taken away after the hardening of the plants.

Analysis

All the experiments were repeated two times. Each treatment had 16 explants divided equally into 4 replications (16 explants = 4 explants × 4 replicates). The regenerants were counted as bulblets if they grew leaves. The other regenerants were considered as bulblet buds only during noting the data at the end of 56 days. The experimental data were analyzed using GLM univariate analysis with IBM Statistical Package For The Social Sciences 20.0 for windows 10. Care was taken to subject the data taken in percentage to Arcsine transformation (Snedecor and Cochran, 1967) before subjecting them to statistical analysis. The Post-hoc tests were performed using Tukey's b Multiple Range Test.

RESULT and DISCUSSION

The biotechnological procedures are serving as an alternative for the conservation of threatened germplasm

with rapid clonal propagation. The present investigations offers an effective *in vitro* propagation protocol for *M. neglectum* on MS basal medium using several BAP+NAA concentrations. The results showed coincidence with the findings of Karamian and Ranjbar (2010) and Karamian et al. (2011) who induced somatic embryogenesis from protoplast culture of *M. neglectum* using a basal medium having several concentrations of BAP+NAA. Fida (2020) has also used BAP+NAA for regeneration from double, triple, quadruple, and quintuple scales and bulblets of *M. neglectum*.

Bulblet regeneration of *M. neglectum* on twin scale explants treated with BAP+NAA enriched MS basal medium

There are some reports of *in vitro* plant regeneration in different ornamental species of genus Muscari using bulb scales (Peck and Cumming 1986; Ozel et. al, 2009, Uranbey, 2010a; Uranbey, 2010b; Nasırcılar et al., 2011; Vaziri et al., 2014; Uzun et al., 2014; Ozel et al., 2015; Ozel and Unal, 2016 and Fida, 2020). The current study reports induction of bulblets on double scale explants using MS basal medium having several concentrations and combinations of BAP+NAA. Thereafter 56 d of the culture of these explants induced 33.33-100 % bulblet regeneration. The results are very similar to the findings of Fida (2020) who induced *M. neglectum*'s bulblet regeneration on 2, 3, and 4 bulbs from 13.33 to 100%. The current study also noted induction of the maximum number of bulblets on explants on MS basal medium having 4 mg L^{-1} BAP-0.5 mg L^{-1} NAA (Fig. 1a). The largest bulblet diameter of 0.3 cm per explant was achieved on 2 mg L^{-1} BAP+ 2 mg L^{-1} NAA. Similarly, Ozel and Unal (2016) reported induction of 8 bulblets on MS basal medium having 0.0454 mg L^{-1} TDZ+ 10.740 mg L^{-1} NAA and 8.25 bulblets on 0.0681 mg L^{-1} TDZ + 2.685 mg L^{-1} NAA using double scales of *M. neglectum.* Care was taken to take experimental data after 8 weeks post-cessation of explants to induce any regeneration of new bulblets or bulblet buds (Table 1). The percentage and number of bulblet buds induction ranged between 0-50% to and 0.00-17.50% in the same order showing a non-significant difference in the performance of culture treatments to induce bulblets excluding control treatments that failed to stimulate any regeneration of any bulblet on the explants. Similar results were also observed by Ozel and Unal (2016) who revealed the induction of 0-16.67% bulblet bud and 0-3.83% bulblets per explant. The bulb buds explants ranged between 75 and 100% per explant. Therefore, it is possible to express that BAP positively promotes the formation of bulblets buds compared to TDZ.

Table 1. Effects of different combinations of BAP+NAA on bulblet induction on double scale explants of *M.neglectum*

Treatments		Percentage (%) of bulblet induction	Mean number of bulblets per explant	Mean Number of bulblets diameter*	Percentage (%) of bulb buds induction[s]	Mean number of bulb buds per explant
BAP mg L^{-1}	NAA mg L^{-1}					
1.0	0.5	33.33	1.63	0.15ab	25.00	1.50
1.0	1.0	83.33	1.88	0.14 ab	50.00	4.92
1.0	2.0	83.33	1.94	0.12 b	33.33	17.50
2.0	0.5	83.33	3.29	0.17 ab	16.67	1.37
2.0	1.0	100.00	3.69	0.14 ab	41.67	2.33
2.0	2.0	50.00	4.5	0.30 a	41.67	8.92
4.0	0.5	66.67	8.00	0.25ab	8.33	8.33
4.0	1.0	100.00	1.25	0.24 ab	25.00	3.17
4.0	2.0	100.00	3.00	0.15 ab	25.00	3.75
MS (control)		100.00	1.00	0.10 b	0.00	0.00

ns nonsignificant, * Means of values in the same column followed by different lower-case letters are statistically different as calculated by Tukeys b test at 0.05 level of significance.

Using primary bulblets induced on double scales as explants under *in vitro* conditions

The primary bulblets (See Table 1, column 4) were used as explants to induce regeneration using 9 different treatments containing several concentrations of BAP+NAA in MS basal medium. The data obtained after eight of the culture treatments revealed that types of plant growth regulator combinations, the basal medium, and influence on the rate of regeneration percentage and multiplication of the species in the genus Muscari that is micro propagated under *in vitro* conditions. These explants stopped regeneration after 10 weeks of culture. Subsequently, it was noted that the differences in initial and final primary bulblets diameters on different culture treatments were very distinguished and conspicuous. The primary bulblets have taken as explants induced a different number of secondary bulbs at different rates. These bulblets have also induced variable percentages and the number of roots with changing lengths (Table 2).

Table 2. Effects of different combinations of BAP+NAA on bulblet induction on primary bulb explants of *M.neglectum*

Treatments		The initial diameter of primary bulblets (cm)*	Final diameter of primary bulblets (cm) ns	The difference in the initial and final bulblet diameters (cm)ns
BAP mg L^{-1}	NAA mg L^{-1}			
1.0	0.5	0.15ab	0.43	0.33
1.0	1.0	0.14 ab	0.36	0.22
1.0	2.0	0.12 b	0.31	0.19
2.0	0.5	0.17 ab	0.32	0.15
2.0	1.0	0.14 ab	0.33	0.19
2.0	2.0	0.30 a	0.48	0.13
4.0	0.5	0.25ab	0.34	0.09
4.0	1.0	0.24 ab	0.35	0.11
4.0	2.0	0.15 ab	0.35	0.20
Treatments				
BAP mg L^{-1}	NAA mg L^{-1}	Secondary bulblet regeneration percentage (%	Mean number of secondary bulblets per explant	Mean number of secondary bulblet diameter (cm) ns
1.0	0.5	72.22 ab	2.41	0.12
1.0	1.0	70.00 ab	3.64	0.22
1.0	2.0	91.67 a	2.00	0.15
2.0	0.5	50.00 ab	1.33	0.17
2.0	1.0	100.00 a	3.04	0.12
2.0	2.0	25.00 ab	1.00	0.21
4.0	0.5	91.67 ab	2.00	0.12
4.0	1.0	83.33 ab	1.00	0.23
4.0	2.0	75.00 ab	1.13	0.15
Treatments		Mean number of roots per explant (%)ns	Mean number of roots per explant	Mean root length (cm)*
BAP mg L^{-1}	NAA mg L^{-1}			
1.0	0.5	63.89	2.03	0.70 ab
1.0	1.0	68.33	3.20	0.73 ab
1.0	2.0	0.00	0.00	0.00 b
2.0	0.5	41.67	0.92	0.43 ab
2.0	1.0	58.83	1.50	0.83 a
2.0	2.0	83.33	2.58	0.63 ab
4.0	0.5	33.33	0.67	0.36 ab
4.0	1.0	66.67	3.83	0.70 ab
4.0	2.0	66.67	2.75	0.46 ab

ns nonsignificant, * Means of values followed by different lower-case letters in a column are statistically different as calculated by Tukeys b test at 0.05 level of significance.

The maximum diameter of 0.48 cm was observed on primary bulblets in 2 mg L^{-1} BAP-2 mg L^{-1} NAA (Fig. 1. b-c), and the difference in the initial diameter at the time of culture and final diameter was measured as 0.09-0.33 cm. The percentage of secondary bulblet induction ranged between 0 and 83.33%, the number of secondary bulblets induced on primary bulblets used as explant was 1.00-3.64. Secondary bulblets diameter was 0.12-0.23 cm. Secondary bulblet induction was between 25.00 and 100%. The percentage of root formation on primary bulblets varied from 0.00 to 83.33 % and their roots changed between 0.00 and 3.83. Rooting was noted on all culture treatments excluding the cultures having 1 mg L^{-1} BAP + 2 mg L^{-1} NAA. The longest roots (0.83 cm) were determined on cultures having 2 mg L^{-1} BAP + 1 mg L^{-1} NAA. These results were coincident with Ozel and Unal (2016)'s study. They noted a variable (0.25 to 0.48 cm) increase on *M. neglectum* bulb diameters

of induced bulblets after another 8 weeks of culture. They compared the difference in initial and final diameters and observed a non-significant increase with a range of 0.02 to 0.23 cm. The final largest bulblet diameter generated on MS basal medium having 0.06811 µM TDZ + 19.74 µM NAA showing variable regeneration of axillary bulblets of 50.00-100.00% on all mother bulblets. They reported 1.25 to 6.11 bulblets induced on the mother explants. The largest count of these axillary bulblets was observed on MS basal medium having 0.0454 µM TDZ + 10.74 µM NAA. They noticed an increased bulblet diameters range of 0.10 to 0.23 cm on all culture treatments. Fida (2020) observed bulblet regeneration on 2, 3, 4, and 5 explants of *M. neglectum* and 100% callus induction on 2 bulb scales explants only on MS basal medium having either of 1 mg L^{-1} BAP + 0.8 mg L^{-1} NAA, 1 mg L^{-1} BAP + 1.0 mg L^{-1} NAA or 1 mg L^{-1} BAP + 1.20 mg L^{-1} NAA. Induction of 1.93 small bulblets per explant with a mean diameter of 0.97 cm was also determined. The researcher has also distinguished the largest bulblet on MS basal medium having 1 mg L^{-1} BAP + 0.4 mg L^{-1} NAA with induction of roots, on all bulblets irrespective of the cultures with axillary bulblets induction range of 2.89-3.38 and adventitious bulblet induction range of 3.00-4.00 adventitious bulblets on three, four and five bulb scales used as explants. Bulblet regeneration studies using BAP + NAA have also been reported in many other Muscari species. Similarly, Ozel et al. (2007) identified the maximum count of 3.33 cm large axillary bulblets of *M. macrocrapum*'s on two-scale explants on MS basal medium having 2 mg L^{-1} BAP + 2 mg L^{-1} NAA. Uranbey (2010) used *M. aucheri* as source plant material. He determined the largest count on 4 scale explants using Nitsch medium supplemented with 2 mg L^{-1} BAP. Nasırcılar et al. (2011) also applied MS basal medium having BAP + NAA using *M. mirum* as a source plant. They realized the highest count of bulblets on 2 bulb scale explants compared to embryos used as explants. The maximum bulblet induction percentage of 23.50 per explant was observed on 4 bulb scale explants using MS basal medium enriched with 4 mg L^{-1} mg L^{-1} BAP and 0.25 mg L^{-1} NAA after 5 months of culture initiation. Uzun et al. (2011) have induced micropropagation using immature embryo explants of *M. muscarimi* as source explant. They noted 59 bulblets per explant using MS basal medium having 4 mg L^{-1} BAP + 0.5 mg L^{-1} NAA after a prolonged culture of 1 year. Azad and Amin (2012) used *M. armeniacum* as an explant source with induction of the highest number of the bulblets on MS basal medium having 4.0 µM BAP + 1.0 µM NAA. Faruq et al. (2019) also induced 5.6 explants using 4.0 µM BA + 2.0 µM NAA.

Rooting of bulbs on MS basal medium and adaptations to the external environment

After 14-21 d of subculture, the primary bulbs developed root initials, which grew to full-length roots (Table 2). They were then transferred to MS basal medium for further development (Table 3). At the end of eight weeks, a significant elongation was observed on the roots of all explants. At the end of 56 d, the variation between the first and final diameter of these rooting bulbs was found to be none significantly different. A comparison between the initial and final diameter and root length revealed significantly different results as shown in Table 3.

Tablo 3. Rooting of *M. neglectum* using MS medium

Treatments		Initial diameter of bulblets (cm)^{ns}	The final diameter of primary bulblets (cm)*	Differences in the initial and final diameter of bulblets (cm)^{ns}	Mean number of roots per explant **	Mean root length (cm)*
BAP mg L^{-1}	NAA mg L^{-1}					
1.0	0.5	0.48	0.73 a	0.24	3.62 b	3.97 b
1.0	1.0	0.36	0.43 ab	0.36	1.85 b	4.93 b
1.0	2.0	0.31	0.32 b	0.05	1.12 b	9.10 ab
2.0	0.5	0.32	0.39 ab	0.04	2.33 b	14.20 a
2.0	1.0	0.33	0.44 ab	0.11	4.08 b	4.98 b
2.0	2.0	0.43	0.49 ab	0.06	7.83 a	5.77 b
4.0	0.5	0.34	0.45ab	0.11	2.30 b	5.65 b
4.0	1.0	0.34	0.41 ab	0.22	2.12 b	5.50 b
4.0	2.0	0.35	0.40 ab	0.10	2.26 b	5.50 b

ns nonsignificant, * Means of values followed by changing lower-case letters in each column are significantly different as calculated by Tukeys b test at 0.05* and 0,01** significance level.

Table 3 reveals that the initial diameters varied between 0.31 and 0.48 cm and the negative effects of growth regulators on the regenerated bulbs were released by transferring them to MS basal medium with 100% rooting (Fig. 1.a). The largest bulblet had a diameter of 0.73 cm that was produced on MS basal medium having 1 mg L^{-1} BAP+0.5 mg L^{-1} NAA. The highest induction of 7.83 bulblets/explant was determined on 2 mg L^{-1} BAP+2 mg L^{-1} NAA (Fig. 1. d-e-f-g). The longest roots of 14.2 cm were observed on bulblets that were induced on cultures having 2 mg L^{-1} BAP+0.5 mg L^{-1} NAA. These bulblets were replaced with pots and their adaptation was ensured (Fig 1.h). The potted bulbs were later transferred to the external environmental conditions in the fields. It has been reported that *M. macrocarpum* and *M. muscarimi* also needed a plant growth regulator-free medium to root (Ozel et al., 2009; Ozel et al., 2016). However, Uzun et al. (2014) observed 5% rooting on *M. muscarimi* with successful acclimatization to the external environment. Nasırcılar et al. (2011) distinguished rooting of *M. mirum* bulbs induced on BAP+NAA using MS basal medium singly. Contrary to these studies, Fida (2020) has rooted *M. neglectum* on an auxin-containing medium. The biggest bulblets in thickness produced on two-scale explants were subjected to rooting using ½ × MS basal medium having 0.5 mg L^{-1} NAA. However, the researcher found a 17% survival rate of the bulblets.

Figure 1. Regeneration rooting and adaptation of *M. neglectum* (a) primary bulblet induction on MS basal medium containing 4 mg L^{-1} BAP + 0.5 mg L^{-1} NAA (bc) 2 mg L^{-1} BAP - 2 mg L^{-1} NAA (defg) 2 mg L^{-1} BAP - 2 mg L^{-1} NAA (h) 2 mg L^{-1} BAP - 2 mg L^{-1} NAA were subjected to rooting on MS basal medium and adapted to the external conditions in soil-filled pots

CONCLUSION

The results of this study meet the objectives planned. Considering the closeness of Turkey to floral markets in the Middle East & Europe and favorable climatic conditions, *M. neglectum* could be easily produced under *in vitro* conditions in a considerable short time with a high rate of success. This research has developed a protocol for commercial propagation and production of *M. neglectum* bulblets.

REFERENCES

Azad, M.A.K., Amin, M.N. 2012. Effects of hormonal and basal nutrient medium on in vitro regeneration of an ornamental plant-Muscari armeniacum Leichtlin. ex Baker. Plant Tissue Culture and Biotechnology, 22(2): 113-126.

Chittenden, F.J. 1956. Dictionary of gardening. Clarendon Press, Oxford, 3: 1329-1331.

Eker, I. Kandemir, A. 2020b. *Muscari sintenisii* Freyn (Asparagaceae)'nin Taksonomik Dirilişi ve Türün

Lektotipifikasyonu. Vineyard Science Journal, 7(3): 12-24.

Eker, I. 2012. *Muscari* Mill. – In: Güner, A. [ed.],A Checklist of Flora of Turkey (Vascular Plants). Nezahat Gökyiğit Botanic Garden and Flora Research Association Publication, İstanbul, 98–100. http://www.bizimbitkiler.org.tr (Access date: 11 07 2021)

Eker, I. 2019a. *Muscari fatmacereniae* (Asparagaceae, Scilloideae), a new species from southern Anatolia. Phytotaxa, 397(1): 99-106.

Eker, I. 2019b. *Muscari pamiryigidii* (Asparagaceae, Scilloideae), a new species from northwestern Anatolia. Phytotaxa, 408(4): 255-266.

Eker, I., Duman, H., Yıldırım, H. 2020a. *Muscari muglaensis* (Asparagaceae, Scilloideae), a new species from southwestern Anatolia. Phytotaxa, 475(4): 267-278.

Eker, İ., Yıldırım, H., Armağan, M. 2019. A new grape yacinth record for the flora of Turkey: Muscari pallens (M. Bieb.) Fisch. Asparagaceae). Vineyard Science Journal, 6(1): 45-53.

Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z., Adıgüzel, N., "Türkiye Bitkileri Kırmızı Kitabı (Eğrelti ve Tohumlu Bitkiler). Türkiye Tabiatını Koruma Derneği Yayınları, Ankara", 1-30 (2000).

Eroğlu Özkan, E.E., Kayıran, S.D., Taşkın, T., Abudayyak, M. 2017. *In vitro* antioxidant and cytotoxic activity of *Muscari neglectum* growing in Turkey. Marmara Pharmaceutical Journal, 22(1): 74-79.

Eroğlu, H. 2020. Morphology, Palinology and Seed Surface Researches on the Taxa of Genus Muscarı Mill. (Asparagaceae) Spreads in Turkey, Ph.D. Thesis, Van Yüzüncü Yıl University, Institute of Science, Van, Turkey.

Faruq, M. O., Shahinozzaman, M., Azad, M., Amin, M. 2019. *In vitro* propagation of a cut flower variety *Muscari armeniacum* Leichtl. ex Bak. through direct bulblet proliferation pathways. Journal of Ornamental Plants, 9(2): 105-114.

Fida, A. 2020. *In vitro* propogation of Muscari neglectum Guss. widely grown in the natural flora of Van region, M.Sc. Thesis, Van Yüzüncü Yıl University, Institute of Natural and Applied Sciences, Van, Turkey.

Goktas, O., Ozen, E., Baysal, E., Mammadov, R., Alma, M. H. 2010. A research on the usage of extracts from two poisonous plants (*Muscari neglectum* Guss. and Gynandriris sisyrinchium (L.) Parl.) as a wood preservative. Wood Research (Bratislava), 55(2): 53-62.

Govaerts, R. 2019. World checklist of Asparagaceae. Facilitated by the Royal Botanic Gardens, Kew. Retrieved April 15, 2019 from http://apps.kew.org/wcsp

He, X.D., Ko, J.A., Choi, J.R., Kim, H.M., Kim, M.J., Choi, S.R., Kim, Y.G., Kim, D.H. Kim, H.S. 2006. Somatic embryogenesis from various parts of *Muscari comosum* var. plumosum. Korean. Journal of Plant Reearchs, 19(3): 427–431.

Jafari, A., Maassoumi, A.A. (2011). Synopsis of *Leopoldia*, *Muscari* and *Pseudomuscari* (Hyacinthaceae) in Iran, with *Leopoldia ghouschtchiensis* sp. nova. In: Annales Botanici Fennici Finnish Zoological and Botanical Publishing Board. 48(5): pp. 396-400.

Karamian R, Sharifzadeh A, Ranjbar M. (2011). Evidence of somatic embryogenesis for plantlet regeneration in *Muscari neglectum* Guss., African Journal of Agriculture Research, 6(14): 3247-3251.

Karamian, R., & Ranjbar, M. (2011). Somatic embryogenesis and plantlet regeneration from protoplast culture of *Muscari neglectum* Guss., African Journal of Biotechnology, 10(22): 4602-4607.

Kayiran, S.D., Özhatay, N., & Kaya, E. (2019). *Muscari tauricum*

(Asparagaceae, Scilloideae), a new species from Turkey. Phytotaxa, 399(2): 109-118.

Kocak, M., Sevindik, B., Izgu, T., Tutuncu, M., & Mendi, Y.Y. (2019). Synthetic Seed Production of Flower Bulbs. In Synthetic Seeds. Springer, Cham.pp: 283-299).

Lim, T.K. (2014). *Muscari neglectum.* In: Edible medicinal and nonmedicinal plants. Springer Science Business Media, Dordrecht, pp 122–125.

Mahboubi, M., & Taghizadeh, M. (2016). The antimicrobial and antioxidant activity of *Muscari neglectum* flower ethanol extract. Herba Polonica, 62(4).

Meydan, İ. (2019). Characterızation of ethanol extract and oil with Gs-Ms of almond (*Amygdalus trichamygdalus*) fruit. Information Technology and Aplication Science, 14 (2): 241-250.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with 277 tobacco cultures. Physiology Plantarum. 15: 473-497

Nasırcılar, A.G., Mirici, S., Karagüzel, Ö. , Eren, Ö., Baktir, İ. 2011. *In vitro* propagation of endemic and endangered *Muscari mirum* from different explant types. Turkish Journal of Botany, 35: 37-43.

Nasrabadi, M., Halimi, M., Nadaf, M. 2013. Phytochemical screeningand chemical composition of extract of *Muscari neglectum*. Middle-East Journal of Scientific Research, 14(4): 566-569.

Ozel Ç.A. 2008. *In vitro* bulblet production of different *Muscari* species, Ph.D. Thesis, Gazi University, Institue of Science and Technology, Ankara, Turkey.

Ozel, C.A., Khawar, K.M., Unal, F. 2007. *In vitro* axillary bulblet regeneration of Turkish yellow grape hyacinth (*Muscari macrocarpum* Sweet) from twin scale explants. Research Journal of Agriculture and Biological Sciences, 3(6): 924-929.

Ozel, C.A., Khawar, K.M., Unal, F. 2015. Factors affecting efficient *in vitro* micropropagation of *Muscari muscarimi* Medikus using twin bulb scale. Saudi Journal of Biological Sciences, 22(2): 132-138.

Ozel, C.A., Khawar, K.M., Arslan, O., Unal, F. 2009. *In vitro* propagation of the golden grape hyacinth (*Muscari macrocarpum* Sweet) from twin scale explants. Propagation of Ornamental Plants, 9(4): 169-175.

Ozel, Ç.A. Ünal, F. 2016. Efficient *in vitro* clonal propagation of *Muscari neglectum* Guss. Ex. Ten using thidiazuron-α naphthalene acetic acid. Turkish Journal of Agriculture-Food Science and Technology, 4(12): 1173-1178.

Peck, D.E., Cuming, B.G. 1986. "Benificial effects of activated charcoal on bulbletproduction in cultures of *Muscari armeniacum*. Plant Cell, Tissue and Organ Culture, 6: 9-14.

Rohollahi, I., Naji, A.M. 2020. Genetic diversity and population structure of wild Persian grape hyacinths (*Muscari neglectum* Guss. ex Ten.) assessed by morphological and molecular markers. Genetic Resources and Crop Evolution, 338(1).

Snedecor, G.W. Cochran, W.G. 1967 Statistical Methods. The Iowa State Univ. Press, Iowa. USA. P. 327-329.

Uranbey, S. 2010a. *In vitro* bulblet regeneration from immature embryos of *Muscari azureum*. African Journal of Biotechnology, 9(32): 5121-5125.

Uranbey, S. 2010b. Stimulating effects of different basal media and cytokinine types on regeneration of endemic and endangered *Muscari aucheri*. Archives Biology of Science, 62(3): 663-667.

Uranbey, S., İpek, A., Caliskan, M., Dundar, E., Cocu, S., Basalma, D., Guneylioglu, H. 2010. *In vitro* bulblet induction from bulb scales of endangered ornamental plant *Muscari azureum*. Biotechnology &

Biotechnological Equipment, 24(2): 1843-1848.

Uzun, S., Parmaksiz, I., Uranbey, S., Mirici, S., Sarıhan, E.O., İpek, A., Özcan, S. 2014. *In vitro* micropropagation from immature embryos of the endemic and endangered *Muscari muscarimi* Medik. Turkish Journal of Biology, 38(1): 83-88.

Vaziri, P.A., Uranbey, S., Sancak, C. 2014. Efficient *in vitro* microprapagation for the conservation of endemic and endangered aucher-eloy grape hyacinth [*Muscari aucheri* (Boiss.) Baker]. Journal of Applied Biological Sciences, 8(1): 80-83.

Yıldırım, Ö. 2020. Keşişbaşı'nın (*Muscari azureum* Fenzl) Research on the seed reproduction of Keşişbaşı. M.Sc. Thesis, Ahievran University, Graduate School Of Science, Kırşehir, Turkey.

Yildirimli, S. 2010. Some new taxa, records and taxonomic treatments from Turkey. The Herb Journal of Systematic Botany, 17(2): 1-114.

Yücel Şengün, İ. Öztürk, B. 2018. Some natural antimicrobials of plant origin, Anadolu University Journal of Science and Technology C- Life Sciences and Biotechnology, 7(2): 256-276.

URL-1.http://www.tubives.com/ (Access date: 14.07.2021)

URL-2. https://www.ipni.org/ (Access date: 12.07.2021)

URL-3. https://wcsp.science.kew.org/qsearch.do (Access date: 12.07.2021)

URL-4. https://www.resmigazete.gov.tr/eskiler/2020/12/20201218-6.htm (Access date: 18.05.2021