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Development of New Sustainable Sahlep Production Methods Using *Ophrys sphegodes* subsp. *mammosa* (Desf.) Soo ex E. Nelson

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ABSTRACT

Tubers of the tuberous orchids have been collected for centuries, and used for medicinal purposes as well as in beverage and ice-cream industry. These orchids (Sahlep orchids) cannot be propagated vegetatively as they generally do not yield more than one tuber in a year. Seeds do not have microscopic endosperms; they have quite small chance of germination and successful establishment. These orchids are not cultivated and are usually collected from natural populations. Therefore, they are under threat of extinction and thus are placed under protection worldwide with national and international treaties. In this study, specimens of the threatened orchid taxa *Ophrys sphegodes* subsp. *mammosa* (Desf.) Soo ex E. Nelson orchid, were stimulated to grow tuber twice in a single vegetation period. Plants' ability to develop new tubers was tested and a new propagation method was proposed for the first time worldwide. Plants harvested at the beginning, middle, and end of flowering period were re-planted. It was observed that the plants harvested at early flowering were able to develop new tubers when they were re-planted in their original places. At the end of their annual life cycle, the number of tubers that were produced by the plants harvested at the three subsequent harvest periods were 2.21, 2.10, and 1.04. Quality of the tubers of the 2nd harvest was also tested in this study to use them as propagation material for the subsequent season, and positive outcomes were achieved. According to the present findings, growth of two tubers in a single vegetation period was achieved for the first time. Possible sustainable cultivation of Sahlep orchids was proved by this new propagation method.

Keywords: Propagation material, Harvest time, Tuberous orchids, Tuber yield.

INTRODUCTION

Tubers of tuberous orchids are harvested and traded from the eastern Mediterranean to the Caspian Sea for the traditional product Sahlep (de Boer *et al.*, 2017; Ghorbani *et al.*, 2017). Turkey has one of the richest orchid flora in the Mediterranean region (Deniz, 2012; Löki *et al.*, 2015). This flora is well documented by Kreutz and Çolak (2009). There are 24 different orchid genera and 187 species and sub-species of the *Orchidaceae* family in Turkey (Tubives, 2018). Of these species, 77 belong to the genus O*phrys*. In other words, *Ophrys* is the richest tuberous orchid genus of Turkey (Löki *et al.*, 2015; Tubives, 2018; Sezik, 2002; GDF, 2018). Among *Ophrys* species, the sub-species *Ophrys sphegodes* subsp. *mammosa* (Desf.) Soo ex E. Nelson has a quite widespread distribution. It is generally widespread in the Aegean, Mediterranean and Black Sea regions of Turkey. This taxon is also widespread in Southeastern Europe, Caucasus, Ukraine, Balkan Peninsula, and Great Britain.

The tuberous orchids (Sahlep) are not cultivated and they are usually collected

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from natural populations (Kreutz, 2002; Molnar *et al.*, 2017a). Excessive collections easily destroy natural population and there is ever-increasing genetic erosion (Kasparek and Grimm, 1999; Molnar *et al.*, 2017b). Collection of tuberous orchids (Sahlep) was banned to prevent destruction and collectors were subjected to heavy fines (Ghorbani *et al.*, 2014; Yaman, 2013). In Turkey, according to 2018 environmental law, Sahlep collectors were subjected to 48.600 TL (about $10.000) fines. Despite all these fines and punishments, wild tuberous orchids (Sahlep) populations are still destroyed by wild-crafters. Statistical data is not available for the collected quantities of Sahlep material as they were illegally collected. However, the information gathered from Sahlep buyers and processing firms revealed that annually 500-600 tons of fresh Sahlep tubers (about 120.000.000 orchids) were used in Turkish markets (Yaman, 2013). There are no alternatives of Sahlep especially in beverage and ice-cream industry and this phenomenon has indicated that illegal collection and commerce of these species will continue (Ar, 2000; Tecimen *et al.*, 2010; Erzurumlu and Doran, 2011, de Boer *et al.* 2017).

All orchid species are included under Annex B of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). A total of 183 countries signed the treaty of CITES (Hagsater and Dumont, 1996; Özhatay *et al.*, 1997). This taxon is listed under Appendix I of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). Tuberous orchids (Sahlep) have two tubers, an old and a new one. The old one is gradually depleted while the new one is developing. Thus, low propagation rates and over-collections from wild populations have already put tuberous orchids (Sahlep) under threat (Tamer *et al.*, 2006; Rankou, 2011). *O. sphegodes* subsp. *mammosa* develops a single tuber for the upcoming year and the old tuber gradually decays. In brief, *O. sphegodes* subsp. *mammosa* is also among the non-cultivated species worldwide.

Tuberous orchids can be cultivated either vegetatively or generatively. However, there are significant obstacles in both methods. The researchers focusing on generative cultivation are trying to develop tubers from the seeds through in vitro methods. Orchid seeds do not have endosperms and they have a primitive embryo (Arditti and Ghani, 2000). Protocorms have been produced from the seeds with in-vitro procedures, but tuber development lasts for many years. In their natural environment, plants propagated from seeds started flowering and produced harvestable-size tubers after at least 4 years (Gümüş, 2009, Tutar *et al.* 2013b).

Bektaş *et al.* (2013) observed during tuber collection phase that there were quite small tubers, probably derived from seed in wild *O. sphegodes* subsp. *mammosa* populations. Our observation on the development of *O. sphegodes* subsp. *mammosa* in the first three years is presented in Figure 1. It was detected that the seedling developed two small leaves and a tiny tuber, 2-3 mm in length, in the first year, and 4 leaves and a larger tuber in the second year, but not a flower. The seed-derived plant flowered in the third year, but did not produce a harvestable-size tuber. Based on these observations, it can be estimated that *O. sphegodes* subsp. *mammosa* could produce a harvestable-sized tuber at the end of the 4th year of its life span. It should be noted, however, that there is no report on the afore-mentioned pattern of reproductive behavior for any of tuberous orchids in the current literature. Sahlep tubers develop a single new tuber throughout the entire growing season and, in this case, it is not possible to gain tubers for commercial purposes.

Literature reviews regarding the obstacles in production processes revealed that plants could survive and develop a second tuber in case the tubers were collected during the flowering period (Tutar *et al.* 2013a). Likewise, Sevgi *et al.* (2012) collected wild *Serapias bergonii* E. G. Camus individuals and moved them to laboratory after

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Figure 1. Development of possibly seed-derived *O. sphegodes* subsp. *mammosa* individuals in the first, second, and third year.

removing their tubers for morphological observations. Interestingly, they observed that the individuals re-produced new small tubers after tarriance in laboratory. Similarly, Tutar *et al*. (2012) observed the regrowth of *Orchis sancta* and *Serapias vomeracea* individuals producing new small tubers after removing their tubers. Kreutz and Çolak (2009) also reported that re-growth could be observed in re-planted orchids of which fresh tubers were removed.

Based on the facts mentioned above and the absence of data on production of *O. sphegodes* subsp. *mammosa* orchids, we aimed to study a new and effective propagation method for *O. sphegodes* subsp. *mammosa* for the first time.

MATERIALS AND METHODS

The research was conducted in the experimental fields of Ondokuz Mayis University, Vocational High School of Bafra, Samsun, Turkey (41° 33′ 46.20” N – 35° 52′ 20.16” E). *O. sphegodes* subsp. *mammosa* plants were collected from the populations growing wild in the same region and mostly located at uncultivated fields of coastal line of Samsun province at the altitudes of 0-100 m.

O. sphegodes subsp. *mammosa* is among the early orchids. Tubers awake in September and develop shoots. Plants get over winter as plantlet with 3-5 leaves and exhibit a fast growth in spring. They blossom from the first quarter of April to the first quarter of May. Plants complete their annual life cycle until the end of May. Throughout the growth period, old tuber decays; the fresh tuber develops for the next generation and reaches the maximum growth at flowering period (Figure 2).

A total of 300 *O. sphegodes* subsp. *mammosa* orchid tubers were collected in May 2016 as plant material. Legal permissions were obtained from the General Directorate of Natural Reserves and National Parks of the Ministry of Forestry and Water Affairs before the tuber collection works.

All the tubers were kept under common storage conditions and they were planted into the field in September of 2016. Plant growth was monitored until April 2017 and routine agricultural practices (watering, weeding etc.) were applied accordingly. Adult individuals of *O. sphegodes* subsp. *mammosa* orchids usually formed 8-10

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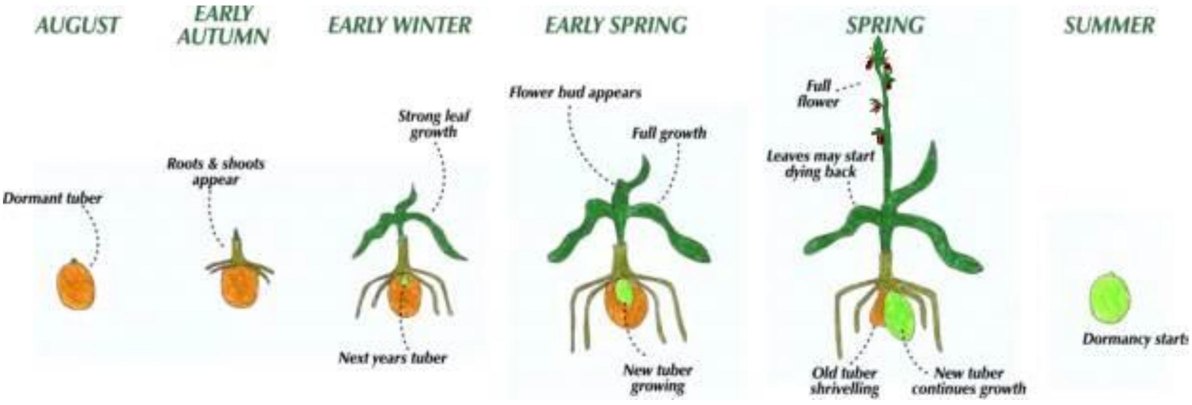

Figure 2. Annual life cycle of Sahlep orchids (Hutchings, 2018).

flowers, the bottom flowers had priority and then flowering advanced towards the top. Flowering lasted about 15-20 days from the first flower incidence to the blossom of the last flower. An individual orchid with the first open flower, another individual with 4 flowers, and another with 9 flowers are shown in Figure 3.

In the present research, the flowering period was divided into three stages. These are: (1) Initiation of flowering (the period in which the first flower blossoms), (2) The middle of flowering (the period in which 4-5 flowers blossom), and (3) End of flowering (the period in which the last flower blossoms). For each harvest period, 100 individual orchids were used. Fresh tubers of the individuals were removed and plants were re-planted into the experimental field and routine agricultural practices were applied. Toward the end of their life cycles, ability of plants to develop a second tuber was determined. Moisture content, fresh and dry weights of the first harvested tubers were recorded. In addition, the time span from harvest to drying was recorded for each flowering stage.

In the second part of the research, suitability of the second tubers to be used as propagation material was tested based on harvest periods. For this purpose, tubers developed by the plants were left until summer and planted into planting trays (small growing medium as seen in Figures 4-5) in August. On Nov 15, their development was recorded.

RESULTS AND DISCUSSION

The first tuber of the plants, pulled out from the soil at different harvest periods, were removed (1st harvest). Harvested plants were then re-planted and cared accordingly. Dates of the first harvest carried out in different flowering stages, mean fresh and dry tuber yields per plant in the first harvest, and life spans passed until plant dried out are shown in Table 1.

Considering the tuber yields per plant in Table 1, it can be concluded that the new tubers usually reach the full-size at full flowering. Tuber yield at the end of the flowering period was found to be 23 and 20% higher than that of the other two periods, respectively. However, we observed that 96% of the plants harvested at the end of flowering were not able to develop new tuber when they were re-planted (Table 2). Our aim was not to produce a single tuber per plant, but to develop a sustainable tuber production method, making possible both commercial utilization and protection of the tuberous orchid populations. Thanks to the newly described method, the tuber obtained at the first harvest can be used as a commercial material.

Data in Table 2 indicate that plants harvested at the beginning and middle of flowering periods have longer life span to re-produce a new tuber. After harvest at the aforementioned periods, plants were able to

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Beginning of flowering | Middle of flowering | End of flowering

Figure 3. Photos of different *O. sphegodes* subsp. *mammosa* orchids at the beginning, middle and end of flowering.

Table 1. Tuber yields per plant in the first harvest and time elapsed until the end of annual life cycle.

Harvest period	Harvest date	Fresh tuber yield per plant (g)	Dry tuber yield per plant (g)	Moisture content (%)	Time elapsed from the 1st harvest to dry-out
Beginning of flowering	13-16 April	3.32	0.63	81.02	33-35 days
Middle of flowering	23-25 April	3.40	0.63	81.50	23-25 days
End of flowering	29 April-2 May	4.09	0.84	79.50	19-20 days

Table 2. Number of tubers in different harvest periods.

Harvest periods	Number of tubers at the 1st harvest	Number of tubers at the 2st harvest	Total number of tubers	Number of tubers per plant
Beginning of flowering	100	121	221	2.21
The middle of flowering	100	110	210	2.10
End of flowering	100	4	104	1.04

develop a new tuber during reproductive cycle. As shown in Table 2, number of tuber per plant was 2.2 and 2.1 for the harvests at the beginning and middle of flowering stages, respectively.

The primary objective of the present research was to increase the number of tuber per plant and to determine value of newly produced tubers as production material. For these purposes, newly developed tubers were planted into growing medium (peat) after storage at room temperature (20±2°C) during three months to test their vigor; and

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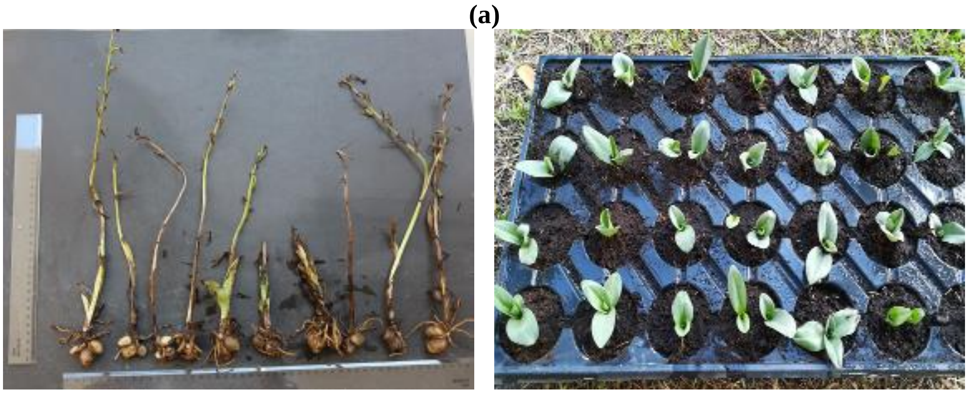
we observed that they exhibited a normal growth.

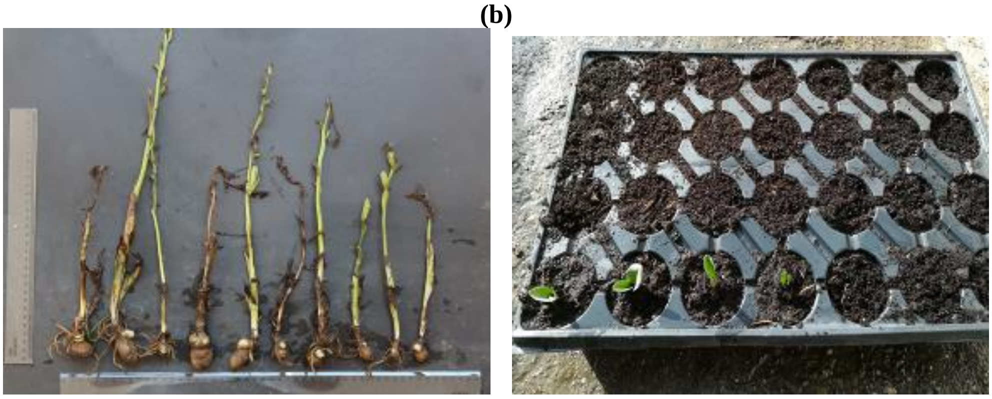
Interestingly, some of the plants of which fresh tubers were harvested at the beginning and middle of flowering developed two new tubers, different in size (Figures 4a-b). These new tubers were planted into the same cell of viol and they yielded two plantlets proving that all the tubers were able to develop a new individual plant regardless of their size.

The re-planted plants of which tubers were harvested at the end of flowering were not able to develop a new tuber because of their remaining short life span. Only 4 of 100 plants harvested at the end of flowering were able to develop a new individual (Figure 4-c). The present findings are in accordance with those of Tutar *et al.* (2012) reporting re-growth of *Orchis sancta* and *Serapias vomeracea* individuals after tuber harvesting at early periods in subsequent seasons. In the present study, *O. sphegodes* subsp. *mammosa*, belonging to the same orchid family, exhibited

(a)



(b)



(c)

Figure 4. Newly developed tubers of the plants harvested (a) at the beginning flowering period (b) at the middle flowering period(c)at the end of flowering period; re-planting and development of these tubers in the next vegetation season.

a significant reproductive performance.

CONCLUSIONS

Plants usually develop alternative reproductive strategies to survive in response to destructive effects of biotic/abiotic environmental factors. Loss of the main tuber providing essential resources to newly emerging plantlet in the next season forces tuberous orchids (Sahlep) to develop new strategies to survive. As reported by Kreutz and Çolak (2009), Sahlep orchids have emergency shoot. Loss of the new tuber while the plant is still active results in awakening of this emergency shoot. Removal of the main tuber has a negative impact on *O. sphegodes* subsp. *mammosa* orchids, which are under the threat of extinction. *O. sphegodes* subsp. *mammosa* orchids normally develop a single tuber throughout the vegetation period, but they can develop new tubers in response to removal of the main tuber at early flowering. This phenomenon can make it possible to develop a new propagation method for tuberous orchids, and in the present paper, it is the first time we have described the new propagation method in details. We advise the farmers who wish to cultivate *O. sphegodes* subsp. *mammosa* orchids that they should harvest tubers at early flowering and re-plant the plants without tuber to produce new tubers. In this way, sustainable Sahlep production could be possible. Further studies to scale up the production of *O. sphegodes* subsp. *mammosa* orchids are currently under way.

REFERENCES

1. Ar, E. 2000. Orchids and the Current Situation of Turkey's Orchids. *Derim*, **17(3)**: 136–152.
2. Arditti, J. and Ghani, A. K. B. 2000. Numerical and Physical Properties of Orchid Seeds and Their Biological Implications. *New Phytologist*, **145**: 376-421.
3. Bektaş, E., Cüce, M. and Sökmen, A. 2013. *In Vitro* Germination, Protocorm Formation, and Plantlet Development of *Orchis coriophora* (*Orchidaceae*), a Naturally Growing Orchid Species in Turkey. *Turk J Bot.*, **37(2):** 336-342.
4. de Boer, H. J., Ghorbani, A., Manzanilla, V., Raclariu, A. C., Kreziou, A., Ounjai, S., Osathanunkul, M. and Gravendeel, B. 2017. DNA Metabarcoding of Orchid-Derived Products Reveals Widespread Illegal Orchid Trade. *Proc. Biol. Sci. Sep.*, **27**: 284 (1863).
5. Deniz, I. G. 2012. Critically Endangered (CR) Orchid Species of Antalya Province of Turkey. XIth IS on Flower Bulbs and Herbaceous Perennials. *Acta Hort.*, **1002**: 37-42.
6. Erzurumlu, G. and Doran, I. 2011. Salep Culture and Salep Orchids in Turkey. *Harran J. Agric. Food Sci*., **15(1)**: 29–34.
7. Ghorbani, A., Gravendeel, B., Naghibi, F. and de Boer, H. 2014. Wild Orchid Tuber Collection in Iran: a Wake-up Call for Conservation. *Biodivers. Conserv.*, **23(11)**: 2749-2760.
8. Ghorbani, A. Gravendeel, B. Selliah, S. Zarre, S. and de Boer, H. 2017. DNA Barcoding of Tuberous Orchidoideae: A Resource for Identification of Orchids Used in Salep. *Mol. Ecol. Resour. Mar.* **17(2)**: 342-352.
9. Gümüş, C. 2009. Investigations for the Reproduction Methods of Some of the Orchid Species (*Orchidaceae*) Used for Obtaining Salep in Western Black Sea Region. Doctoral Thesis, Ankara University, 221 PP.
10. Hagsater, E. and Dumont, V. 1996. *Orchids: Status Survey and Conservation Action Plan*. IUCN, Gland Switzerland and Cambridge, UK, 173 PP.
11. Hutchings, J. 2018. *Growing Hardy Orchids*. Laneside Hardy Orchid Nursery, Annual Cycle of the Anacamptis. http://www.lanesidehardyorchids.com/growing_hardy_orchids.htm
12. Kasparek, M. and Grimm, U. 1999. European Trade in Turkish Salep with Special Reference to Germany. *Econ. Bot.*, **53(4)**: 396-406.
13. Kreutz, C. A. J. 2002. Turkey's orchids. Salep, Ice Cream and Massacre. In: Yesil Atlas", PP. 98-109.
14. Kreutz, C. A. J. 2009. *Turkey Orchids*. ISBN: 6054015078, First Edition, (Ed.): Hüseyin Çolak, Rota Publications, 855 PP. (in Turkish)

15. Kreutz, C. A. J. and Çolak, H. 2009. *Turkey Orchids*. Rota Publications. ISBN: 6054015078. 855 p. (in Turkish).
16. Loki, V., Tokolyi, J., Süveges, K., Lovas-Kiss, Á., Hurkan, K., Sramko, G. and Molnár V, A. 2015. The Orchid Flora of Turkish Graveyards: A Comprehensive Field Survey. *Willdenowia*, **45(2)**: 231-243.
17. GDF. 2018. "*Orchid Action Plan 2014-2018*". Republic of Turkey General Directorate of Foresty Publications, 36 PP. Downloaded on 13 April 2018.. https://www.ogm.gov.tr/ekutuphane/Yayinlar/Salep%20Eylem%20Plan%C4%B1.pdf
18. Molnár, V., Nagy, T., Löki, V., Süveges, K., Takacs, A., Bodis, J. and Tökölyi, J. 2017a. Turkish Graveyards as Refuges for Orchids against Tuber Harvest. *Ecol. Evol.*, **7(24)**: 11257-11264.
19. Molnar, A., Suveges, K., Molnar, Z. and Löki, V. 2017b. Using Traditional Ecological Knowledge in Discovery of Rare Plants: A Case Study from Turkey. *Acta Societatis Botanicorum Poloniae*, **86(3)**: 1-10.
20. Özhatay, N., Koyuncu, M., Atay, S. and Byfield, A. 1997. *Türkiye'nin Doğal Tıbbi Bitkilerinin Ticareti Hakkında bir Calışma*. ISBN 975-96081-9-7, Natural Life Protection Association Publications, İstanbul, PP. 103-106. (in Turkish)
21. Rankou, H. 2011. *Ophrys sphegodes* subsp. *mammosa*. The IUCN Red List of Threatened Species 2011: e.T165287A5999979. Downloaded on 13 April 2018. http://www.lanesidehardyorchids.com/growing_hardy_orchids.htm
22. Sevgi, E., Altundağ, E., Kara, Ö., Sevgi, O., Tecimen, H. B. and Bolat, I. 2012. Relations between Morphological Characteristics and Community Structure of *Serapias bergonii* e.g. Camus Species in Çanakkale-Biga. *In Proceedings of the Turkey 2. Orchid and Sahlep Workshop*. 25-26 April 2012. İzmir, Turkey, PP. 157-168.
23. Sezik, E. 2002. Turkish Orchids andS. *Acta Pharmaceutica Turcica*, **44**: 151–157.
24. Tamer, C. E., Karaman, B. and Copur, O. U. 2006. A Traditional Turkish Beverage: Salep. *Food Rev. Int.*, **22**: 43–50.
25. Tecimen, H. B., Sevgi, O, Kara, Ö., Sevgi, E., Altundağ, E. and Bolat, I. 2010. *Türkiye Salep Türlerinin Sorunları ve Öneriler*. ISSN: 1300-8575, Ministry of Environment and Foresty Publications No: 373, **10(2).** (in Turkish)
26. Tubives. 2018. *Turkish Plants Data Service*. http://www.tubives.com/.
27. Tutar, M., Çiçek, F., Sarı, A. O., Bilgiç, A. and Yıldız, Ö. 2012. The Culture of Some Orchid Species Distributed in Aegean Region. *In Proceedings Turkey 2. Orchid and Sahlep Workshop*, 25-26 April 2012, İzmir, Turkey, PP. 301–315.
28. Tutar, M., Sarı, A. O., Parlak, S. and Çiçek. F. 2013a. Vegetative Production in Salep Orchids. *In Proceedings XI Ecology and Environment Congress*, 1-4 October 2013, **88** PP.
29. Tutar, M., Parlak, S., Sarı, A. O. and Çiçek, F. 2013b. Production from Seeds in Salep Orchids. *In Proceedings XI Ecology and Environment Congress*, 1-4 October 2013, Samsun, Turkey, 89 PP.
30. Yaman, K. 2013. Regulations Relating to the Salep and Its Trade in Turkish Official Gazette Archives from 1920 to the Present. *J. History Culture Art Res.*, **2(1)**: 172-180.

روش های نوین و پایدار تولید ثعلب با استفاده از *Ophrys sphegodes* subsp. *mammosa* (Desf.) Soo ex E. Nelson

ا. کالیسکان، د. کورت، و س. سیراک

چکیده

غده های ارکیده غده ای برای قرن ها جمع آوری میشده و برای اهداف دارویی و مصرف در صنایع نوشیدنی و بستنی مورد استفاده بوده است. این ارکیده ها (ارکیده ثعلب) به صورت رویشی(غیر جنسی) قابل تکثیر نیست زیرا آنها معمولا بیشتر از یک غده در سال تولید نمی کنند و بذر آنها فاقد آندوسپرم های میکروسکوپی است که بخت کمی برای سبز شدن و استقرار گیاه دارند. این ارکیده ها کاشت نمی شوند و معمولا از جمعیت های طبیعی جمع آوری می شوند. بنابراین، آنها در تهدید انقراض قرار دارند و در سطح جهانی با قرار دادهای ملی و بین المللی تحت حمایت می باشند. در این پژوهش، نمونه هایی از رده ارکیده تهدیدشده یعنی *Ophrys sphegodes* subsp. *mammosa* (Desf.) Soo ex E. Nelson orchid تحریک به دو نوبت غده سازی در یک دوره رشد شد. توانایی گیاه برای تولید غده جدید مورد آزمون قرار گرفت و برای اولین بار در سطح جهانی روشی جدید برای تکثیر پیشنهاد شد. گیاهانی که در ابتدای گلدهی،در وسط، و در آخر دوره گلدهی برداشت شده بودند مجددا کاشته شدند. چنین مشاهده شد که گیاهان برداشت شده در ابتدای گلدهی چنانچه در محل اولیه مجددا کاشته شوند قادر به تولید غده های جدید بودند. در آخر چرخه زندگیشان، تعداد غده های تولید شده در سه مرحله برداشت پشت سرهم به ترتیب برابر ۲/۲۱، ۲/۱۰، و۱/۰۴ بود. کیفیت غده های برداشت دوم نیز آزموده شد تا از آنها به عنوان مواد قابل تکثیر در فصل بعدی استفاده شود. از این آزمون نتایج مثبت به دست آمد. بر پایه یافته های این پژوهش، برای اولین بار، رشد دو غده در یک دوره رشد با موفقیت همراه بود. به این ترتیب، امکان کاشت پایدار ارکیده ثعلب با این روش جدید تکثیر به اثبات رسید.

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