Anadolu Tarım Bilimleri Dergisi
Anadolu Journal of Agricultural Sciences
http://dergipark.gov.tr/omuanajas

Araştırma/Research

Anadolu Tarım Bilim. Derg./Anadolu J Agr Sci, 35 (2020)
ISSN: 1308-8750 (Print) 1308-8769 (Online)
doi: 10.7161/omuanajas.712434

Describe the long-term development of *Ophrys apifera* Huds. salep orchids in natural flora

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Geliş/Received 01/04/2020 Kabul/Accepted 28/04/2020

ABSTRACT
Salep orchids are annual plants. Planted salep tuber produces a slightly larger tuber than itself and completes its life. Microscopic seeds without endosperm do not germinate under normal conditions. Therefore, they cannot be reproduced and are protected species. There is no data on the growth and development of salep orchids in natural flora over the years. *Ophrys apifera* Huds. species was selected as plant material in this research. The germinated sprouts of this species have been identified and observed. Plants observed in their natural habitats for five years, morphological characteristics were measured at the end of each vegetation year. According to the obtained data, germinated seed completed its life as a tiny two-leaf plant capable of producing 0.19 grams of tuber in the first year. In the following years, each tuber has completed its life by producing larger tubers. At the end of five years, the plants reached an average length of 43.1 cm and 4.01 g of salep tubers were formed.

Keywords:
Annual development
Flower
Growing speed
Orchids
Tuber

Ophrys apifera Huds. Salep Orkidelerinin Doğal Florada Uzun Süreli Gelişiminin Tanımlanması

ÖZET
Salep orkideleri tek yıllık bitkilerdir. Dikilen herbir salep yumrusu, kendisinden biraz daha büyük bir yumru üretir ve ömrünü tamamlar. Endospermsiz mikroskobik tohumlar normal koşullar altında çimlenemez. Bu nedenle çoğaltılamazlar ve korunan türlerdir. Doğal florada salep orkidelerinin yıllar içinde büyümesi ve gelişmesi hakkında veri yoktur. Bu çalışmada *Ophrys apifera* Huds. türü bitki materyali olarak seçilmiştir. Bu türün çimlenmiş filizleri tanımlanmış ve gözlemlenmiştir. Beş yıl boyunca doğal yaşam alanlarında gözlemlenen bitkilerin, her vejetasyon yılı sonunda morfolojik özellikler ölçülmüştür. Elde edilen verilere göre, çimlenmiş tohum ilk yılda 0.19 gram yumru üretebilen iki küçük yapraklı bir bitki olarak ömrünü tamamlamıştır. Sonraki yıllarda, her yumru daha büyük yumru/lar üreterek devam etmiş, beş yılın sonunda bitkiler ortalama 43.1 cm uzunluğa ulaşmış ve 4.01 g salep yumrusu oluşturmuştur.

Anahtar Sözcükler:
Büyüme hızı
Çiçek
Orkideler
Yıllık gelişim
Yumru

1. Introduction

The Orchidaceae genus is the second largest genus in the world. With newly found and hybrid species, their numbers are increasing day by day (Arditti and Ghani, 2000). Within the genus, species that are grouped as terrestrial orchids and produce tubers under the ground are known as salep orchids. Tubers of salep orchids have been used as medicinal plants for centuries (Kasparek and Grim, 1999). Today, it is mostly consumed in ice cream making and as hot drink in Turkey (Sezik, 2002; Hossain, 2011; Gorbani et al., 2014).

Orchids produce thousands of seeds in the form of oval. The common feature of all orchids is that they all have very small dust-like microscopic seeds. The most important feature that distinguishes orchids from other seed plants is the absence of endosperm in their seeds. It has the smallest known seeds in the plant kingdom (Mitchell, 1989). In addition, embryos consist of relatively few cells (40-50). Another noteworthy issue in the seed structure is that the parts that will form the plant parts such as root, stem and shoot after germination are not shaped. This condition is called morphological dormancy. In short, primitive embryo and endosperm are missing seeds (Baskin and Baskin, 1998; Cig et al., 2018).

The salep orchids can produce at least 8-10 seed capsules and tens of thousands of seeds in these capsules. However, seeds as small as dust particles and lacking endosperm cannot survive by themselves and it is impossible for them to sprouting and germinates. In germination stage, seeds cannot grow without support from a different living organism (Sezik et al., 2007; Bektas et al., 2013).

Due to its primitive and microscopic seeds, many seeds sprinkled by orchids have little chance of germination (Utami et al., 2017). Germination of seeds is very slow development and takes many years. It is known that germinated seedling completed the vegetation process with one or two small leaves in the first year.

In the following years, the growth of plants continues and they acquire the ability to form flowers. In this process, the plants continue to massively grow up to a certain stage. The process from seed germination to flowering maturity is called maturity period. Because only plants that can reach generative maturity can produce flowers and produce seeds. How long this time will take depends on the genetic characteristics of the species (Sezik, 2002; Sezik et al., 2007).

Seed germination in in-vitro medium and plant development from protocorms have been achieved worldwide, but the developmental processes of the obtained seedlings have not been followed in the following years (Sezik, 2002; Caliskan et al., 2019).

Tissue culture with in vitro studies are mostly limited to species-based germination studies. Here, some researchers who make observations in natural environments can be included. For example, Tutar et al. (2013) stated that germinating seeds from seed production in salep orchids can reach flowering size after 3-4 years. In a study, germinated seeds of leaves and tubers have occurred after many years reported. According to Sezik (2002), the average shortest period is 2-4 years. However, there is no source as to which type of salep orchid has completed its adulthood and in how many years it has developed.

Among the orchids, the genus *Ophrys* has a high variety with more than one hundred species (Duvey et al., 2008). One of the most common species of the genus *Ophrys* is *Ophrys apifera* Huds. and is called Bee orchid because of its flower shape. The Bee Orchid, *Ophrys apifera* Huds. belongs to the group apifera which is a Mediterranean species. This species extends to Central Asia, Europe, North Africa and the Caucasus (Delforge, 2006; Addam et al., 2015; Szatmari, 2016). This species is among the common species, especially coastal areas, especially in Turkey (Caliskan et al., 2018).

In this study, it was determined that the sprouts of *Ophrys apifera* Huds. species orchid, which were determined to germinate and exit in their natural environment. The obtained sprouts were followed in their natural environment. The growth and development status of the plants, which were followed for 5 years, were revealed. However, no source has been found about the growth and development of the species for many years.

2. Material and Methods

The study material was *Ophrys apifera* Huds. species orchid. It is one of the most common species seen in the province of Samsun where the survey was conducted. During the field trips conducted in November-December 2014, new sprouts were observed in the areas where the species were found to be grown from seed around the adult plants.

These sprouts, which are the test material, were separated from the adult plants and collected together with their own soil in their natural environment and put under protection to complete the first vegetation year (Figure 2a, 2b and 2c).

Salep orchids form a very small tuber with a few small leaves in the first year of germination. This tiny tuber completes the growing process in the next vegetation by producing a new tuber larger than itself. This process continues until the new tuber reaches a certain size. The old tuber roots and disappears at the end of vegetation. In other words, orchids are annual plants. Each year there is a larger plant and tuber development than the previous generation.

a **b** **c**

Figure 1. Sprouts growing from spilled seeds around adult plants.
Şekil 1. Yetişkin bitkilerin etrafına dökülmüş tohumlardan büyüyen filizler

Although germination of salep orchid seeds has been achieved in vitro, there is no literature on the developmental status of these seeds over the years.

In this study, five-year growth performance of germinated seeds was demonstrated. For this purpose, the plants were grown in their natural environment for five years and some morphological features were tested at the end of each year. In order to summarize the annual development status of the plants, the following observations were taken in ten samples at the end of each vegetation year.

Plant height (cm), number of leaves (per plant), leaf length (cm), leaf width (cm), total biomass (g), the wet weight of all biomass), number of flower (per plant), tuber width (cm), tuber length (cm), fresh yield of tuber (g), number of root (per plant) were determined and evaluated as experimental parameters at the end of growing period.

a **b** **c**

Figure 2. Germinated sprouts.
Şekil 2. Gelişmeye başlamış filizler.

3. Results and Discussion

As with all orchids, they have to be fed with the help of another living organizm during the germination stage of their first stage of life. They are fungi from the mycorrhiza group that help germinate the orchard seeds and provide the necessary nutrients (Bonnardeux et al., 2007; Jacobsone, 2008; Bektas et al., 2013; Rasmussen et al., 2015; Herrera et al., 2017). When examined in Figure 2a, it is seen that the germinated and the first leaves of the orchids do not have roots. This proves that the seed is fed by the fungi. Salep orchids are annual plants. However, each new generation produces more biomass and a larger tuber than its parent.

As with other tuberous orchid species, seeds of *Ophrys apifera* Huds. complete the first year as small plants when they germinate. After the first year, the new generation reaches greater mass values than the previous generation. Asymbiotic germination of seeds was performed in in vitro studies, but no data were revealed about the growth status of the shoots that were produced for years. In this study carried out in order to eliminate the lack of information, plants that germinated in their natural environment were used. In this study, annual growth status of plants which were followed for five years in their natural environment were recorded and development performance was determined.

In this study conducted for five years; important morphological features were determined at the end of each vegetation year. The obtained morphological features are shown in Table 1 and Figure 3. The morphological characteristic of the samples are examined graphically and the results are shown in Figure 4. When the examined all data of morphological characteristics (plant height, number of leaves, leaf length, leaf width, total biomass, number of flower,

tuber width, tuber length, and fresh yield of tuber) have got second-degree polynomial curves. But, number of roots has got linear curve. R^2 values changed between 0.89-0.99. The minimum R^2 belongs to plant height and the maximum R^2 belongs to fresh yield of tuber.

3.1. First year

Plants germinated around September and seen on the soil surface during the November-December period started to turn yellow on 25 May 2015. Morphological measurements were taken in order to summarize the developmental performances during this period. In the first year, which can be called as germination year, all the individuals that were included in the study, *Ophrys apifera* Huds. species, formed two tiny leaves and one root. The first leaf is slightly larger than the next leaf. The average leaf length was 3.9 cm and the average leaf width was 0.7 cm the first year of the fresh weight of tuber produced only 0.19 grams (Figure 3a). In their study on the 4-year development period of *Dactylorhiza romana* (sebast.) soo. which is in the group of tuberous orchids, Caliskan and Kurt (2019) found that this species had 1 leaf in the first year, 0.8 cm and 5.8 cm of leaf width and height respectively, and tuber width-length was 0.5 cm respectively. cm and 1.5 cm, tuber wet weight was reported to be 0.25 g.

a **b** **c** **d** **e**

Figure 3. a: First year development status, b: Second year development status, c: Third year development status, d: Fourth year development status, e: Fifth year development status

Şekil 3. a: Birinci yıl gelişim durumu, b: İkinci yıl gelişim durumu, c: Üçüncü yıl gelişim durumu, d: Dördüncü yıl gelişim durumu, e: Beşinci yıl gelişim durumu

Table 1. Morphological characteristics obtained at the end of each vegetation year
Çizelge 1. Her vejetasyon yılı sonunda elde edilen morfolojik özellikler

Morphological Features	Years				
	1st year	2nd year	3rd year	4th year	5th year
Plant height (cm)	-	-	30.3	37.5	43.1
Number of leaves (per plant)	2	3	4	6	6
Leaf length (cm)	3.9	6.7	6.9	7.5	7.4
Leaf width (cm)	0.7	1.2	1.3	1.5	1.6
Total biomass (g)	0.51	2.68	5.54	10.4	11.7
Number of flower (per plant)	-	-	4-5	5-7	9-10
Tuber width (cm)	0.6	1.1	1.3	1.6	1.7
Tuber length (cm)	0.7	1.3	1.6	2.6	2.5
Fresh yield of tuber (g)	0.19	1.12	1.98	3.11	4.01
Number of root (per plant)	1	4	4-5	7	9

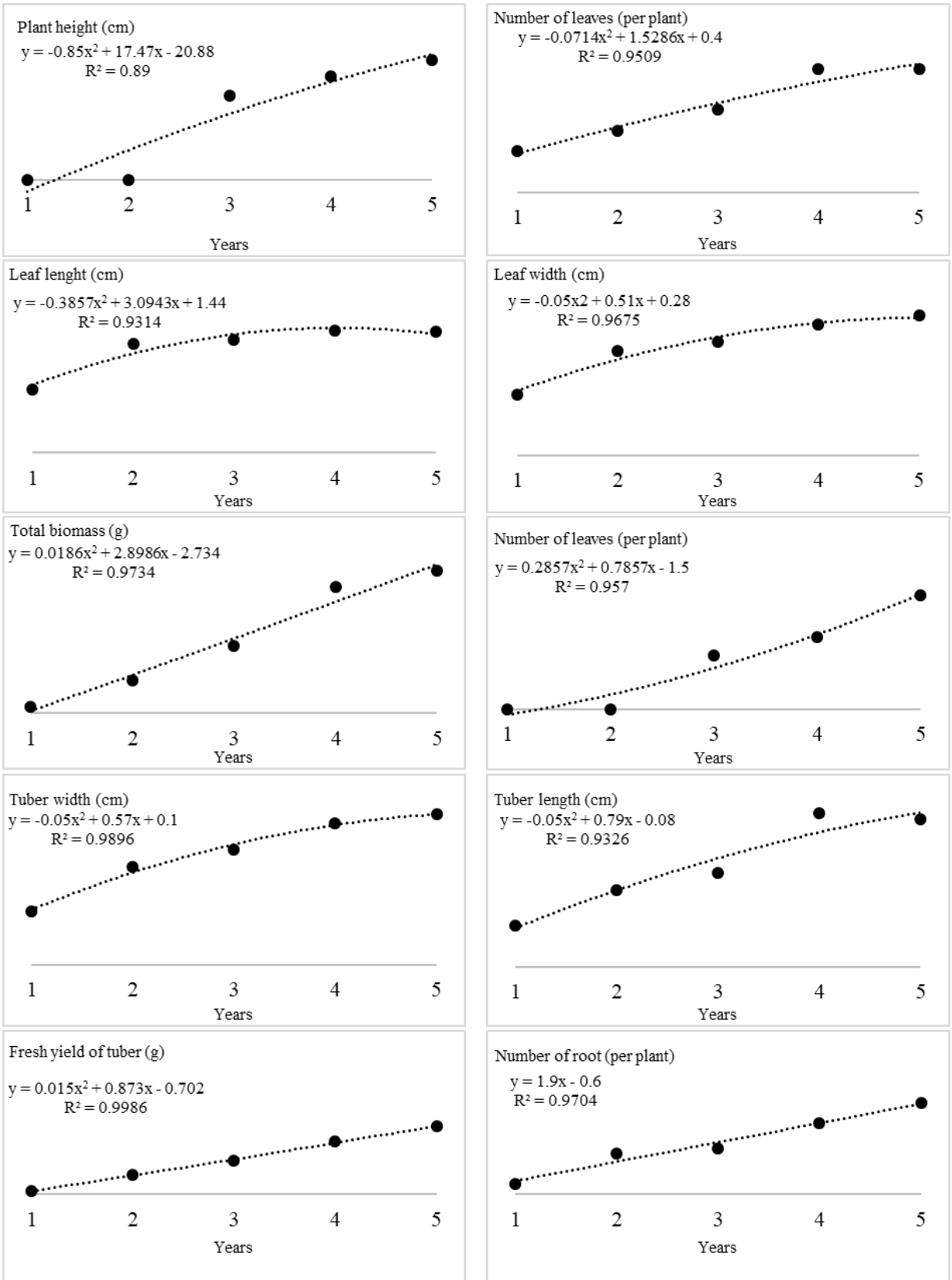

Figure 4. Mathematical change of morphological characteristics and R^2 values.
Şekil 4. Morfolojik karakterlerin matematiksel değişimi ve R^2 değerleri

After morphological measurements, obtained tubers were stored in a cool, dry warehouse and in paper bags in the summer period in order to protect them from natural pests. As is known, the orchid tubers spend the summer season as dormant (Tutar et al., 2012). These tubers, which were developed by seed germination and obtained in the first growing season, were planted in their natural environment on September 1, 2015.

3.2. Second year

The tubers obtained in the first year were planted in their natural environment and taken under protection in September. The plants that developed under natural conditions continued their vitality and development in the environment where they were located for about nine months. When the edges of the leaves began to turn yellow, it was understood that they completed their development and they were dismantled on May 25, 2016 for morphological measurements. Plants developed under natural conditions have continued their vitality and development in the environment where they are located for about nine months. When the edges of the leaves began to turn yellow, it was understood that they completed their development and they were dismantled on May 25, 2016 for morphological measurements. In the second year vegetation period, the plants produced 3 leaves and 4 roots on average. At the end of the vegetation, the average weight of the tubers in the second year was 1.86 g (Figure 3b). Plant height values were not taken in the first two years. Because, in these years, there are no above-ground parts except the leaves, there has been no flower development. Caliskan and Kurt (2019), for similar reasons, did not take plant length measurements in the *Dactylorhiza romana* (sebast.) soo. species for the first two years. However, they reported that tuber weight increased to 0.99 grams at the end of the second year.

3.3. Third year

Tubers stored for 3 months during the summer were planted on September 5, 2016 to determine the third vegetation year performances. At the end of the third year, 4-5 flowers were observed for the first time. As it is known, the time to reach flowering is called maturity process and in *Ophrys apifera* Huds. species the maturity process is determined as 3 years in their natural environment. To date, there is no record of the flowering time of salep orchids. In this study, the first flowering of *Ophrys apifera* Huds. species was determined to take place in the 3rd year. In the third year, the average plant length was 30.3 cm, the average leaf length was 6.9 cm and the leaf width was 1.3 cm. The fresh tuber weight was 1.98 g and there were 4-5 pcs root in plants (Figure 3c). In the third year of the *Dactylorhiza romana* (sebast.) soo. species, 6-leaf, 16.3 cm plant length, tuber width-length 1.8 cm and 1.9 cm 2.30 g tuber weight reached respectively (Caliskan and Kurt, 2019).

3.4. Fourth year

Tubers were harvested in the third year and they were planted on September 7, 2017 for the fourth vegetation period controls. At the end of nine months of vegetation, the plants were harvested on 29 May 2018 from their environment. According to the measurements, morphological values of plants continued to increase. The plant height was 37.5 cm, leaf length was 7.5 cm, and biomass weight was 10.4 g per plant. The weight of the new tubers reached to 3.11 grams. The plant we observed produced 7-8 flowers in the observed plants (Figure 3d). Similarly, in the study using *Dactylorhiza romana* (sebast.) soo. species, plant height was 24.5 cm, leaf width was between 0.7 - 1.3 cm, leaf width and leaf length was between 6.9 - 9.1 cm, leaf size was 8 leaves, tuber width was 2 cm, tuber length was 2.4 cm, and tubers weighing 3.11 g were developed (Caliskan and Kurt, 2019).

3.5. Fifth year

In the fifth year of the study, the mass increase in plants continued. The average plant height was 43.1 cm, the number of flowers per plant was 9-10 pieces and the weight of the biomass per plant was 11.7 g and 4.01 grams of biomass was the new tuber weight (Figure 3e).

4. Conclusion

Considering that a single orchid orchid produces hundreds of thousands of seeds, it is understood that the survival of the orchid seeds is very low. Seeds that have the chance to germinate in nature complete their development as a tiny plant in the first year. In this study, it is understood that the plants that follow the development of *Ophrys apifera* Huds. species for 5 years can form flowers in the third year and the number of flowers increases due to the mass increase in the following years. The findings obtained are the first data presented for the species. It is also noteworthy that the size of the tuber increases with each passing year. As long as their genetic capacity allows, this condition continues until the tubers reach a certain size in orchid orchids. The main tuber completes its life by producing a slightly larger new tuber. Determining the genetic capacity of the species in terms of size can take decades. In this study, it is understood that when the tubers developed in the first year (0.19 g) were planted, they formed approximately 6 times larger tubers and reached 1.12 g tuber weight. When these tubers were planted for the third year of vegetation, they produced 1.76 times their own weight. In the fourth year of the trial, the tubers gave new tubers with a weight of 1.57 times

more than their own. The fourth year tuber formed 1.32 times its own weight. As can be seen, there is a rapid increase in proportional tuber size in the first years and a slowdown in the rate of increase in the following years. This continues as far as the genetic capacity of the species allows. However, the number of years of genetic capacity reached in terms of tuber weight is unknown in salep orchids. As can be understood from the morphological features, each tuber planted can produce a slightly larger tuber than itself. In the first years of development, the growth rate is proportionally higher and gradually slows down. This study summarizes the five-year development status. Observations will continue in the following years and the second five-year development will be revealed.

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