Pak. J. Bot., 53(3): 1051-1056, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-3(30)

A NEW SUSTAINABLE PRODUCTION METHOD FOR THE SAHLEP ORCHID *SERAPIAS ORIENTALIS* (GREUTER) H. BAUMANN & KUNKELE

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Abstract

Effective production of tuberous orchids (sahlep orchids) from *Orchideceae* family has not yet been achieved. For that reason, all the species of this family are placed under protection worldwide with national and international treaties. In the present study, it was aimed to describe a sustainable production method for *Serapias orientalis* and plants were forced to produce two tubers in the same growing season. For this purpose, 100 plants were cultivated and their fresh tubers were harvested at the beginning of flowering (first harvest). The plants whose fresh tubers were removed were re-planted, thus they completed their growing season. It was observed that the re-planted plants produced new tubers in one month period. Some of them produced more than one tubers and a total of 110 new tubers were produced (second harvest). Finally, 100+110=210 tubers were obtained from 100 plants in one growing season. Productivity of all the tubers was tested in the next growing season and positive results were observed. In conclusion, the present results indicate that *Serapias orientalis* has the ability of producing tubers twice in the same growing season and it is possible to cultivate this plant via the tubers sustainably. The method, described here for the first time, was termed as "dismantle and re-plant".

Key words: Tuberous orchids, *Serapias orientalis*, new production method

Introduction

Terrestrial orchids producing tuber are termed as sahlep orchids and their tubers have been collected from wild populations of Turkey, Iran, Greece, Iraq and Azerbaijan without cultivating. Sahlep flour is an indispensable stabilizer for food industry, especially ice-cream production. Tuberous sahlep orchids traditionally have been consumed for centuries as a food, restorative, tonic, and aphrodisiac (Kasparek & Grimm, 1999; Arditti & Ghani, 2000; Sevgi *et al.,* 2012a; Altundag *et al.,* 2012). Those orchids were mentioned for the first time in B.C. 350 by Greek philosopher Theophrastus who named these plants as "orchis" due to their tubers and mentioned their medicinal values. Famous old physicians such as Dioscorides and Avicenna described tuberous orchids as aphrodisiac and roborant agents (Yonzone *et al.,* 2008; Ghorbani *et al.,* 2014; Endersby, 2016). Before coffee became widespread, sahlep was consumed to a large scale in Europe, especially in England and it was serviced together with bread and butter in beverage houses serving sahlep specially (Tamer *et al.,* 2006). In Tanzania, Zambia and Malawi, tubers of terrestrial orchids have been used in making a staple food, called chikanda (Kreziou *et al.,* 2015). Sahlep is also used to make a sexual tonic and aphrodisiac in Indian Ayurveda medicine and in Unani medicine in India and Pakistan (Ghorbani *et al.,* 2014).

Sahlep orchids have been over collected from wild populations for a long time by insusceptible wild crafters who dismantle the whole plant after getting their tubers at flowering. In this way, the wild plants both lose their new tubers from which they will reproduce in the next year and cannot produce seeds. This phenomenon has turned up the pressure on wild populations of tuberous sahlep orchids (Sezik, 2002). Seeds of sahlep orchids are also problematic, so they do not have endosperms and then they have quite small chance of germination and successful establishment (Kreutz, 2002; Tecimen *et al.,* 2010). Besides, the formation of tubers which are big enough to reproduce has lasted three years at least, if the seeds could germinate and form a new plant.

All the hitches raised the topic of protecting sahlep orchids in their own natural flora. In the treaty of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), came into force in 1975 and have signed by 183 countries now, sahlep orchids are listed as "endangered species". Wild gathering and trade of approximately 400 sahlep orchids are banned in the countries, signing CITES (Cites, 2017). Turkey signed this treaty in 1996 and has imposed a fine of 48.000 TL (9.000 US $) to sahlep wild crafters (Yaman, 2012, 2013; Caliskan, 2018). In spite of all the precautions and punishments, millions of wild sahlep orchids have collected annually for their valuable tubers in word wide (Kreutz, 2002; Tecimen *et al.,* 2010; Tutar *et al.,* 2013). The information, gathered from sahlep processing firms and buyers indicated that annually 500-600 tons of fresh sahlep tubers (about 120.000.000 orchids) were traded illicitly in Turkish markets (Caliskan, 2018). Several man-made factors such as urbanization, transforming the natural habitats of orchids into farmland and overgrazing have also threatened wild sahlep orchid populations (Atik *et al.,* 2010).

It is possible to cultivate the sahlep orchids vegetatively and generatively to some extent, but it should be noted that there are significant handicaps in both methods. Tuber development lasts for many years if seeds could germinate in nature. Besides, plants have yielded only one new tuber in growing season making difficult vegetative production. Thus, researchers have focused on developing seed originated tubers by using *In vitro* methods but, they have not described so far an effective production protocol from seed to tuber in economic sense.

Among sahlep orchids, *Serapias orientalis* (Greuter) H. Baumann & Künkele is a distinct species with its high

adaptation ability and it is prevalent under temperate zone, especially in Greece, Portugal, Spain, Western France, Algeria and Tunisia (Hertel & Kreutz, 2010; Wilson, 1986). This species is widespread around coastline in Turkey where approximately 120 species of tuberous orchids have been used to produce sahlep (Sezik, 1984; 2002; Kreutz & Çolak, 2009; Arslankaya, 2012).

Literature reviews regarding production of sahlep orchids revealed some promising results. Sevgi *et al.,* (2012b) collected wild *Serapias bergonii* E.G. Camus individuals and moved them to laboratory after removing their tubers for morphological observations. Interestingly, they observed that the individuals re-produced new small tubers after tarriance in laboratory. Kreutz & Colak, (2009) observed that tuberous orchids whose fresh tubers were removed could produce seed and additional tubers when they were re-planted in their original places. Tutar *et al.,* (2012) observed the re-growth of *Orchis sancta* and *Serapias vomeracea* individuals producing new small tubers after removing their tubers.

The results lead us to believe that a new vegetative production method can be developed for sahlep orchids. Thus, it is the first time we have described a new and effective propagation method for the sahlep orchid *S. orientalis* in the present study.

Materials and Methods

Tubers of the sahlep orchid *S. orientalis* which is prevalent in Turkey and temperate zones of the word was used as plant material. 100 *S. orientalis* tubers were collected from the populations growing wild in uncultivated fields of coastal line of Samsun province, Turkey. Official permissions were provided from the General Directorate of Natural Reserves and National Parks of the Ministry of Forestry and Water Affairs before the tuber collection works.

S. orientalis tubers have sprouted in September under ecological conditions of Middle Black Sea region of Turkey and spent the winter as seedling. The plants growing during winter season have produced the new tuber from which they will reproduce in the next year in March-April and flowered in the first half of May. Flowering has followed a manner from down to up and lasted 4-6 weeks with intervals of 2-3 days. Each plant has produced 8-10 flowers. Flowering and seed formation have lasted during May. Growing season is ended in the first half of June. Thus, seeds have maturated at the end of 30-40 days between beginning of flowering and drying of plants.

Each plant has produced one new tuber within its growing season and this new tuber has reached its maximum size at flowering. The main tuber has decayed towards the end of growing season. The new tuber from which the plant will reproduce in the next year is dormant until beginning of September. Typical lifecycle of sahlep orchids is shown in Fig. 1.

New tubers were harvested at the beginning of flowering in May 10, 2017; the plants without new tubers were re-planted into the same place and routine agricultural practices were applied until growing season ended in compliance with the new production method we described here. The plants were dismantled in June 12, 2017 when aerial parts of the plants dried out and the new tubers plants reproduced were photographed.

Suitability of the new tubers, produced by the re-planted plants to be used as propagation material was tested. For this purpose, these tubers were left in cool and shade place during three months and planted into planting trays (small growing medium) in September 01, 2017 (Fig. 2).

Results and Discussion

The *S. orientalis* tubers sprouting in September 5-10, 2016 continued to develop routinely until May 10, 2017 and then flowered. 100 new tubers, produced by 100 tested plants were described as first harvest tuber and stored after dismantled. The re-planted plants without fresh tubers completed their life cycle in June 12, 2017 when the aerial parts were dried. Then, the experiment was ended and it was observed that the 100 plants whose new tubers were removed exhibited five different reactions in response to re-planting (Table 1).

As shown in Table 1, the re-planted plants produced 110 new tubers within last month of their growing season and a total of 210 tubers were produced together with first harvest tubers in one growing season. The new 110 tubers were stored under normal storage conditions and planted into planting trays to test their vigor and suitability as propagation material in September, 2017. It was observed that all the tubers sprouted in healthy way and could be used as propagation material. The reaction manners of re-planted plants in Table 1 were detailed below.

1. Main tuber decayed and three new tubers were produced (7x3=21 new tubers): 7 of 100 tested individuals produced three new tubers after removing their fresh tuber in response to re-planting. The new tubers had different sizes and the smallest one was 5 mm in length and 3 mm in width. It was observed in October 15, 2017 that all the three tubers continued to develop routinely (Fig. 3a, b).

2. Main tuber decayed and two new tubers were produced (12x2=24 new tubers): 12 of 100 re-planted plants produced two new tubers (Fig. 4a). Development status of the vigorous new tubers in October 15, 2017 is shown in Fig. 4b.

3. Main tuber decayed and one new tuber was produced (28x1=28 new tubers): 28 of 100 re-planted plants produced one new tuber (Fig. 5a) and it was observed that the new tubers were vigorous as shown in Fig. 5b.

4. Main tuber was plump and one new small tuber was produced (36x1=36 new tubers): 36 of 100 re-planted plants produced one new tuber smaller than that of plants exhibiting number three reaction in response to re-planting (Fig. 6a). It is interesting to note that main tubers appeared like vigorous in this group of re-planted plants. Generally main tubers have shriveled up during

plant ontogenesis and decayed entirely at the end of growing season. Exceptionally, main tubers of the 36 plants could retain their vigorous appearances leading us to suppose that they were alive. However, these main tubers shriveled during experiment when stored in summer season and it was revealed that they were not alive. The new smaller tuber, however, grew in healthy way (Fig. 6b).

5. Main tuber was plump or decayed and no new tuber: 18 of 100 re-planted plants did not produce new tuber and they completed the growing season with their plump or decayed main tubers. As mentioned above, the live-appearing main tubers shriveled entirely when stored in summer season during experiment and these 18 plants exhibited no development after re-planted.

There are many national and international treaties regarding threatened species, the most comprehensive of which is CITES, signed by 183 countries. All the sahlep orchids are put under protection in accordance with the treaty of CITES due to the fact that these species can be propagated neither generatively nor vegetatively. Actually, dismantling the tuber from which plant will reproduce in the next year has stressed sahlep orchids out and triggered some physiological mechanisms which resulted in induction of emergency shoot as reported by Kreutz & Colak (2009). In this period, plants were forced to produce new tubers to survive by removing their fresh tuber. Likewise, in the present study, 100 fresh tubers were provided by 100 experimental *S. orientalis* plants in first harvest and then 7, 12 and 64 ones of tested 100 plants produced 3, 2 and 1 new additional tuber, respectively when re-planted after their fresh tuber was removed. Thus, we managed to produce a total of 210 tubers in the same growing season in the sahlep orchid *S. orientalis*. The results are the first in worldwide and can lead up effective production of sahlep orchids. Likewise, the new production method we described here makes possible to get 210/100=2.1 tubers per plant in the same growing season while sahlep orchids produced only one tuber under normal circumstances.

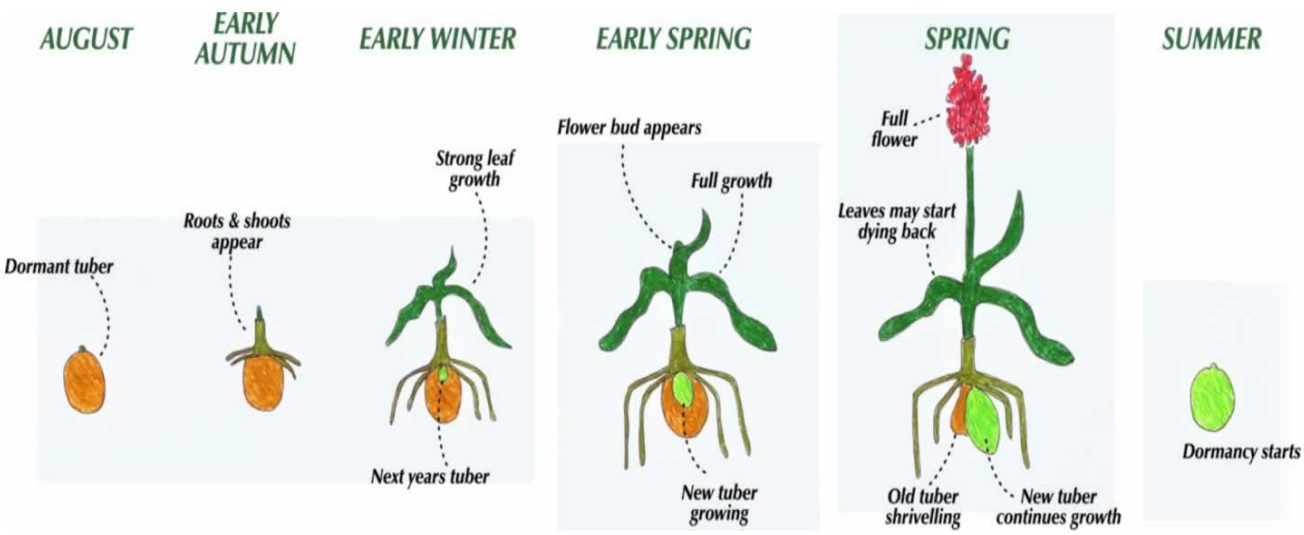

Fig. 1. Typical lifecycle of sahlep orchids (Anon., 2017).

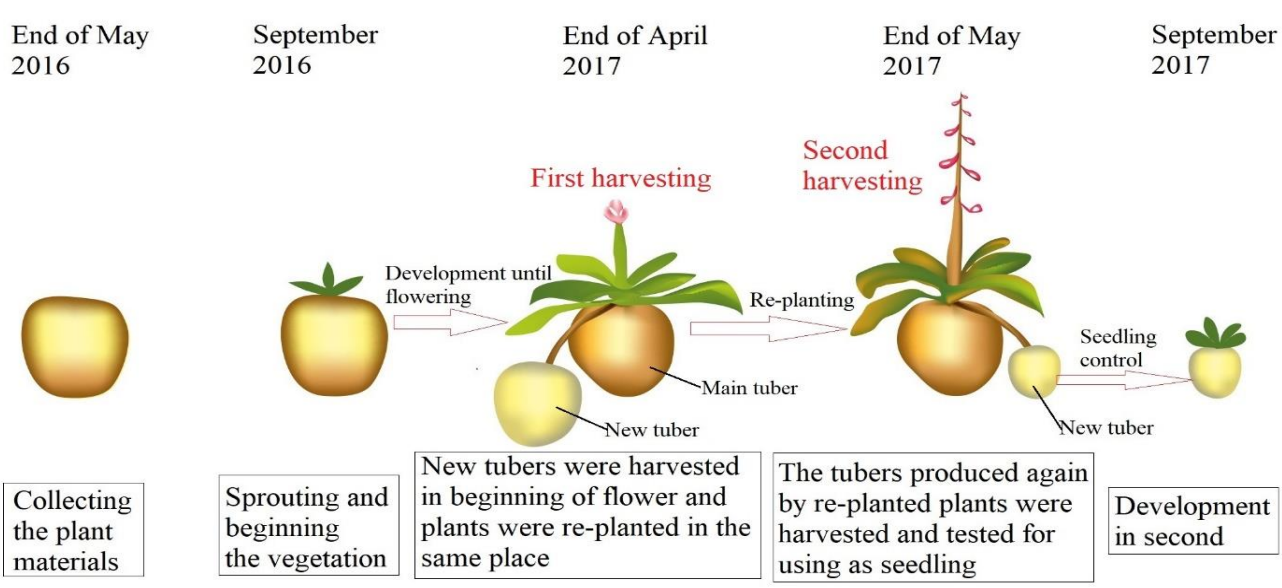

Fig. 2. Illustrated form of the new production method for *S. orientalis*, described in the present study.

Table 1. Reactions of the *S. orientalis* plants whose new tubers were removed in response to re-planting.

S.No.	Reaction manners	Number of the plant exhibiting this response in the second harvest	Number of tubers in the second harvest
1.	Main tuber decayed and three new tubers were produced	7	7 x 3 = 21
2.	Main tuber decayed and two new tubers were produced	12	12 x 2 = 24
3.	Main tuber decayed and one new tuber was produced	28	28 x 1 = 28
4.	Main tuber was plump and one new small tuber was produced	36	36 x 1 = 36
5.	Main tuber was plump or decayed and no new tuber	18	0
	Total	**100**	**110**

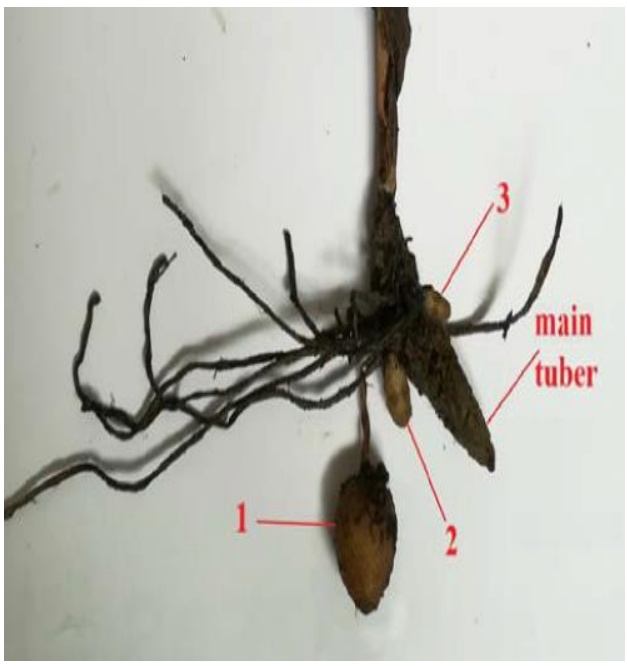

Fig. 3a. The re-planted individual whose main tuber decayed and producing three new tubers.

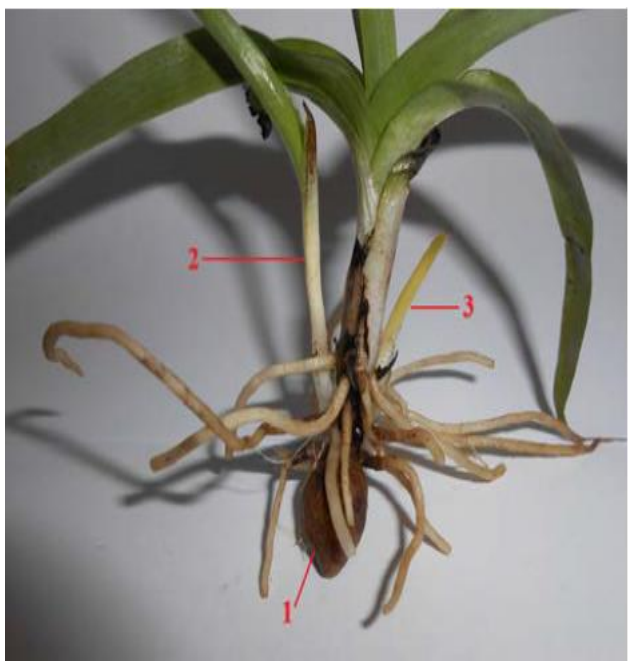

Fig. 3b. Development of three new tubers after re-planting.

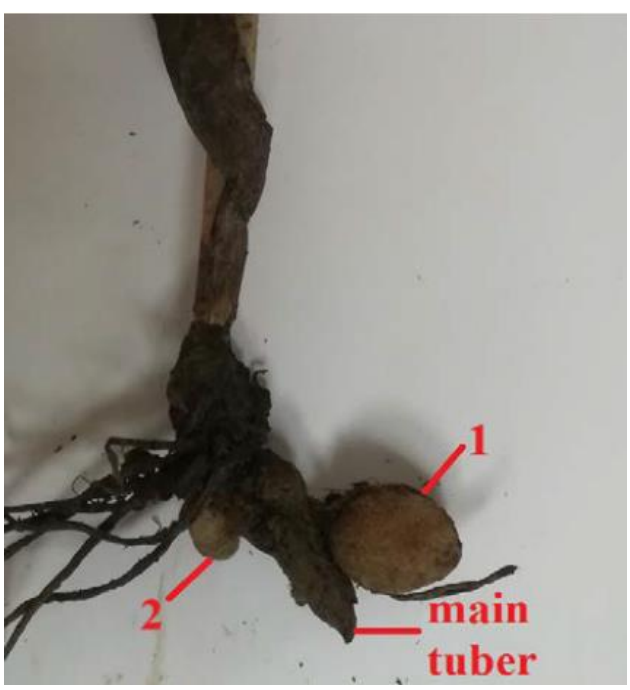

Fig. 4a. The re-planted individual whose main tuber decayed and producing two new tubers.

Fig. 4b. Development of two new tubers after re-planting.

Fig. 5a. The re-planted individual whose main tuber decayed and producing one new tuber.

Fig. 5b. Development of the new tuber after re-planting.

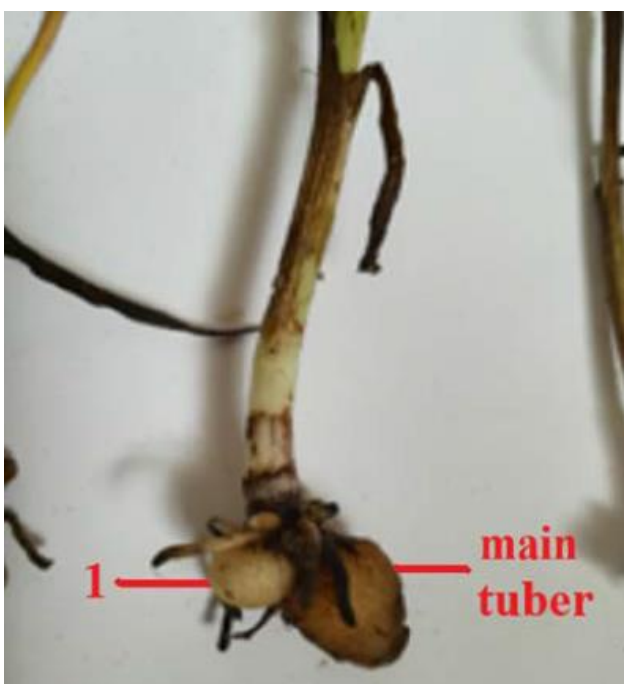

Fig. 6a. The re-planted individual whose main tuber looks well and producing one new smaller tuber

Fig. 6b. Development of the new smaller tuber after re-planting

Conclusion

The new production method, described here proved experimentally that the sahlep orchid *S. orientalis* could be propagated under culture conditions. According to the new method, we named as “dismantle and re-plant” first harvest tubers could be traded and propagation of the orchids continued in the same production area via the re-planted plants after their fresh tubers were removed. The present results also indicated that it is possible to get commercial sahlep tubers annually with the method without injuring wild plant populations. Thus, it could be possible both to prevent the devastation of sahlep orchid’s populations and to describe a new income channel for local farmers. However, it should be noted that the new production method was achieved by using only 100 *S. orientalis* plants and the preliminary results need to be validated by the field studies with a reasonable number of individuals. In this sense, our further studies on large scale up production of *S. orientalis* as well as several other sahlep orchids under field conditions are currently underway.

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(Received for publication 18 April 2019)