

Bulb Induction on Madonna Lily Stems and Three Years Growing Performance of the Bulblets

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ABSTRACT

Currently, Madonna lily (*Lilium candidum* L.) is considered as one of the most important ornamental and perfumery plants. Madonna lily is currently propagated via seed or bulb scales. However, seed derived plants reaches sexual maturity at the end of five years and flower production is interrupted in vegetative propagation via bulb scales because fertile bulbs are needed as source of scale. To overcome the two obstacles, stems of mature plants were used as material for bulb production in the current study. For this purpose, the stems, obtained from plants at the end of flowering were stored in cardboard boxes under the conditions of darkness, normal temperature and pressure (20°C and 1 atm) during four months. At the end of this period, it was observed that 14 bulbs per stem formed on the stored stems. The stem derived bulbs were cultivated in pots and were observed to determine their development performances and some morphological characters until reaching flowering during three years. An increase of 226% in bulb weight, 51.2% in bulb diameter and 58.9% in bulb circumference were observed at the end of the three years. In this study, a new and effective method for Madonna lily propagation was described for the first time.

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Akzambak Bitki Gövdesinde Soğan Üretimi ve Yavru Soğanların Üç Yıllık Gelişim Performansı

ÖZET

Günümüzde Akzambak (*Lilium candidum* L.) en önemli süs ve parfümeri bitkilerinden biri olarak kabul edilmektedir. Akzambak halihazırda tohum veya soğan pulları ile çoğaltılmaktadır. Ancak tohumdan elde edilen bitkiler ancak beş yılın sonunda eşey olgunluğuna ulaşır ve vejetatif çoğaltmada çiçek üretimi, pul kaynağı olarak verimli soğanlara ihtiyaç duyulduğundan kesintiye uğrar. Bu iki engeli aşmak için bu çalışmada soğan üretim materyali olarak olgun bitkilerin sapları kullanılmıştır. Bu amaçla çiçeklenme sonunda bitkilerden elde edilen saplar dört ay boyunca karanlık, normal sıcaklık ve basınç koşullarında (20°C ve 1 atm) karton kutularda saklanmışlardır. Bu sürenin sonunda depolanan gövdelerde gövde başına 14 adet soğan oluştuğu görülmüştür. Saptan türetilen soğanlar saksılarda yetiştirilmiş ve üç yıl boyunca çiçeklenmeye ulaşmaya kadar gelişme performanslarını ve bazı morfolojik özelliklerini belirlemek amacıyla gözlemlenmiştir. Üç yılın sonunda soğan ağırlığında %226, soğan çapında %51.2 ve soğan çevre uzunluğunda %58.9 artış gözlenmiştir. Bu çalışmada Akzambak üretimi için yeni ve etkili bir yöntemi ilk kez tanımlanmıştır.

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INTRODUCTION

The plants of *Lilium* (Liliaceae) are one of the oldest plants humans have used for centuries with different

purposes. Historical records have indicated that these plants were cultivated thousands of years ago in Mediterranean basin (Fornaris et al., 2011). Ancient

Egyptians used lilies as wreath in funeral ceremonies and Romans used them as ornament to decorate their palace gardens (Mammadov et al., 2017). Furthermore, lily gardens were also mentioned in Sumerian tablets, prepared about 5000 years ago (Ozen et al., 2012; Dhiman et al., 2018). Today, lilies are cultivated as ornamental plants throughout the world, as well as used as important perfumery plants. *Lilium* species also have significant pharmacologic activities as antitumor (Yuan et al., 2019), anti-inflammatory (Ma et al., 2017), antioxidant, antibacterial (Hui et al., 2019), hypoglycemic (Xiao et al., 2014), anti-fatigue and anti-hypoxia (He et al., 2009) activities that were attributed to the bioactive constituents of bulbs namely, saponins, sterols, alkaloids, polysaccharides, glycerol glycerides, phenylpropanoids and flavonoids (Wang et al., 2019).

Lilium genus consists of 120 species growing mainly wild in temperate zone of Northern Hemisphere and is represented in Turkey by the presence of seven species, one of which is *Lilium candidum* L. (Demir and Celikel, 2017). This perennial bulbous plant grows in sparse populations on rocky slopes and maquis shrubland of Mediterranean basin. Its distribution areas include Balkan countries, Greek islands, Lebanon, Palestine, Israel, Syria and the coastal regions of southwestern and western parts of Anatolia. In Turkey, *L. candidum* spreads naturally in territory of coastal cities of Mediterranean and Aegean geographic regions, namely Aydın, İzmir, Mersin, Muğla, Antalya, Balıkesir, Çanakkale, İstanbul. It prefers especially maquis shrublands and rocky shades which receive sufficient sunlight but are not exposed to sun directly as habitat (Kahraman, 2014, 2015; Ocak et al., 2014).

Thanks to its large and shining white flowers, *L. candidum* has been accepted as the symbol for innocence in worldwide and is named as Madonna lily (Fornaris et al., 2011). In Turkey, this plant is known under the local names “akzambak, beyaz zambak, mis zambak, desti zambağı ve bey zambak” (Ocak et al., 2014). Madonna lily has been used traditionally in folk medicine for the treatment of inflamed and purulent wounds, ulcers, skin inflammations, burns and various injuries since ancient times (Pieroni, 2000; Zaccai et al., 2020). Like the other lilies, it includes many of chemical compounds with a wide range of bioactivities. However, it has been recognized in worldwide as an ornamental and cut flower in recent years. Today, the most popular using way of Madonna lily is production of ornamental plants. It is also an economically important perfumery plant with its pleasant odor (Royandazagh and Pehlivan, 2016; Patocka and Navratilova, 2019).

Today, *L. candidum* is one of the species that are in danger of extinction. Harvesting its bulbs is forbidden and heavy fines are levied from ones collecting the

wild bulbs. In addition, this prominent flower is listed in the species under protection according to CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Export for *L. candidum* bulbs is only permitted in cultivation areas. For that reason, it is necessary to describe cultivation practices for this species with the aim of protecting the flora and increasing its trade value.

Madonna lily is currently propagated via seed and bulb scales generatively or vegetatively (Alam et al., 2013; Kahraman et al., 2017). Seeds are dormant in the winter season and produce one small bulblet by germinating in spring. The number of bulb scale and bulb diameter increase significantly in consecutive years and the bulb has reached the capacity of flowering approximately at the end of five years (Akcal and Kahraman, 2016). Thus, propagation via seeds takes a longer time than propagation via bulb scales (Balge et al., 2020). In vegetative production, the bulb scales that are obtained only from large bulbs are used as material and flower production is interrupted as a result. The large bulbs, consisting of many scales with various dimensions are divided into their scales and each scale produces a new plant. Bulblet of the scale-derived plantlet develops enough to blossom after three years (Altan and Burun, 2017; Royandazagh et al., 2015). Several efforts have also been undertaken for in vitro production of *L. candidum* (Khawar et al., 2005; Bakhshaie, 2016; Mazor et al., 2021).

Alternatively, stems were used as propagation material instead of seeds and bulb scales in the present study. To the author's knowledge, there is no report on bulb formation on stems and development performance of the stem derived bulbs in the current literature. Thus, we stored the stems, obtained from plants at the end of flowering in cardboard boxes and observed bulb formation on these stems during storage period. Then, we cultivated the stem-derived bulbs in pots during three consecutive years and determined their performance as propagation material. In this way, it is the first time we have described here a new and effective method for Madonna lily propagation.

MATERIALS and METHODS

In the present study, it was aimed to induce bulbs on stems and to describe development performance of the stem-derived bulbs until they reached flowering. The study was conducted out in two phases; firstly, bulb formation on stems and secondly determination of growth and development performances of the stem derived bulbs during cultivating in pots. The stems that were used as material were kindly provided by lily producers from the Bafra district of Samsun province, Turkey.

The climate of the Bafra district, characterized by hot

and dry summers, cool and rainy winters, reflected typically general climate characters of Black sea region and is semi-humid. Most precipitation falls in the autumn and winter months. The spring and summer months are relatively less rainy. The snow cover stays in the region for a short time or for some years it does not snow at all. The first frost occurs in November and the last frost in April. The average temperature is 12.5 °C as mean of long years, the highest average temperature was recorded in July and August (22.1-22.5 °C) and the lowest average temperature was recorded in January and February (2.1-3.4 °C). On average, relative humidity rate is 65.4% and total amount of annual precipitation is 520.2 mm (Yildirim, 2020).

In the first stage, stems of plants at the end of flowering were harvested on June 06, 2017. Total of 100 stems were stored in cardboard boxes under the conditions of darkness, normal temperature and pressure (20 °C and 1 atm) for four months. Then, the bulbs formed on stems at the end of storage were counted and separated.

In the second stage, the stem derived bulbs were transferred into the pots, 15 cm in diameter and 3 liters in volume on October 02, 2017 to observe the growth and development performances of individuals until reaching flowering during three years. The pots were filled with a mixture of 80% soil + 15% commercial peat + 5% burnt barn manure. The study was conducted out in the open under semi-shaded

conditions during three consecutive years. As experimental data, bulblet number per stem, root length (cm), root weight (g), bulb weight (g), bulb diameter (cm), bulb circumference (cm), scale number per bulb, and total fresh weight were recorded at the end of each vegetation period. Fresh weight of basic leaf (g) and basic leaf number per plant in the first two years and plant height (cm), leaf number per plant, stem weight (g) and stem diameter (cm) in the last year were also recorded.

RESULTS and DISCUSSION

Bulblet production phase (June 01, 2017 – October 01, 2017)

The stems, picked from mature plants at the end of flowering were stored as mentioned previously and bulblet formation was observed on these stems after four months of storage (October 01). The stems in cardboard boxes before storage are shown in Figure 1a and the bulblets formed on these stems are shown in Table 1b. The stem-derived bulblets were separated from stem tissue by bistoury and photographed after sorted from big to small for exhibition (Figure 1c-d).

According to the results, the tested stems produced a number of large and small bulblets. As shown in Figure 1, bulblet formation occurred on stems from bottom to top and lower bulblets were larger, but upper ones were smaller. It is also noteworthy to note





Figure 1. The stored stems in cardboard boxes (A), bulblets before (B) and after (C-D) separated from stems.

Şekil 1. A; Bitki saplarının muhafaza edildiği ambalajlı durumları, B; Yavru soğancıkların gövdeden ayrılmadan önceki durumları, C-D; Yavru soğancıkların sap üzerinde ve koparıldıktan sonraki durumları

Table 1. Data for the plants with one or two basal leaves in first growing season.

Çizelge 1. Birinci vejetasyon yılını bir veya iki bazal yaprakla tamamlayan bitkilere ait veriler

| | Mean | SE | SD | Min. | Max. | CI (95%) |
|--|------|------|------|------|------|-------------------|
|  Basal leaf fresh weight (g) | 0.24 | 0.01 | 0.02 | 0.21 | 0.27 | 0.229 ≤...≤ 0.257 |
| Basal leaf length (cm) | 7.30 | 0.26 | 0.82 | 5.79 | 8.33 | 6.708 ≤...≤ 7.884 |
| Basal leaf width (cm) | 2.36 | 0.06 | 0.21 | 2.06 | 2.74 | 2.211 ≤...≤ 2.505 |
| Root length (cm) | 2.65 | 0.07 | 0.21 | 2.35 | 2.97 | 2.502 ≤...≤ 2.801 |
| Root weight (g) | 0.12 | 0.00 | 0.01 | 0.10 | 0.14 | 0.114 ≤...≤ 0.130 |
| Bulb weight (g) | 1.29 | 0.05 | 0.15 | 1.01 | 1.47 | 1.178 ≤...≤ 1.395 |
| Bulb diameter (cm) | 1.66 | 0.04 | 0.14 | 1.40 | 1.87 | 1.558 ≤...≤ 1.761 |
| Bulb circumference (cm) | 3.10 | 0.10 | 0.31 | 2.73 | 3.63 | 2.872 ≤...≤ 3.322 |
| Scale number per bulb | 3.35 | 0.08 | 0.26 | 3.00 | 3.79 | 3.168 ≤...≤ 3.540 |
| Total fresh biomass (g) | 1.70 | 0.05 | 0.17 | 1.41 | 1.96 | 1.585 ≤...≤ 1.824 |
|  Basal leaf fresh weight (g) | 0.35 | 0.01 | 0.03 | 0.31 | 0.40 | 0.334 ≤...≤ 0.374 |
| Basal leaf length (cm) | 7.10 | 0.25 | 0.80 | 5.64 | 8.10 | 6.527 ≤...≤ 7.671 |
| Basal leaf width (cm) | 2.58 | 0.06 | 0.19 | 2.30 | 2.90 | 2.444 ≤...≤ 2.722 |
| Root length (cm) | 5.74 | 0.14 | 0.45 | 5.09 | 6.44 | 5.420 ≤...≤ 6.070 |
| Root weight (g) | 0.28 | 0.01 | 0.03 | 0.23 | 0.31 | 0.262 ≤...≤ 0.299 |
| Bulb weight (g) | 2.30 | 0.09 | 0.27 | 1.80 | 2.63 | 2.104 ≤...≤ 2.491 |
| Bulb diameter (cm) | 2.25 | 0.06 | 0.19 | 1.90 | 2.54 | 2.114 ≤...≤ 2.390 |
| Bulb circumference (cm) | 4.30 | 0.14 | 0.44 | 3.79 | 5.03 | 3.984 ≤...≤ 4.608 |
| Scale number per bulb | 4.17 | 0.13 | 0.42 | 3.49 | 4.78 | 3.867 ≤...≤ 4.470 |
| Total fresh biomass (g) | 3.02 | 0.09 | 0.30 | 2.51 | 3.47 | 2.809 ≤...≤ 3.234 |

*SE: Standart error, SD: Standart deviation, CI: Confidence interval

that the bulblets started to root before separated the stems. This is especially true for the robust bulblets from bottom part of stems (Figure 1). Bulblet number per stem varied with 7-12 and the tested stems produced 14 bulbs on average. Eken and Sirin (2018) searched the most suitable growing media for different *Lilium* spp. and reported 8.3-17.5 bulblets per stem depending on species. Main bulb bigness, stem thickness and suitability of growing medium are thought to affect bulblet formation. It is obvious that large main bulbs have thick stems and, as shown in Figure 1, have produced higher number of bulblets with a larger diameter. Likewise, Herlina and Winarto (2019) tried lily bulbs with different bigness and reported that main bulb bigness affected yield, yield parameters and various plant characters directly. Similar results were also reported by Arslan *et al.* (2019).

Phase for transformation the bulblets to reproductive individuals (3 years, 2017-2019)

Growth performance for the first year (October 01, 2017 – May 25, 2018)

In the second phase of the study, the stem derived bulbs were cultivated in pots and observed until flowering during three years. As shown in Figure 1, these bulbs varied with their largeness. Accordingly, the plants they produced also varied with their dimensions. It was observed that 33, 27, 18 and 7% of the tested bulbs produced 1, 2, 3 and 4 basal leaf, respectively at the end of first year of cultivation in pots. Some pictures of the first-year plants and data for the morphologic observation and measurements tested are shown in Table 1 and 2. First year planted bulbs thrived in accordance with their largeness and larger bulbs produced higher number of scales. The stem derived bulbs with single scale produced bulbs with 3-8 scales in first year. These bulbs varied in terms of diameter (1.40-3.87 cm), weight (1.01-9.15 g) and circumference (2.73-8.89 cm). Besides, these bulbs produced the plants, 1.41-12.69 g in fresh biomass and these plants provided the leaves, the

smallest and biggest of which were 2.06 x 5.79 cm and 2.78 x 10.02 cm, respectively in dimensions (Tables 1-2). The first vegetation period was ended with leaf drying in June 10 and the harvested bulbs were stored for the second-year cultivation.



Growth performance of the bulbs in the second year (September 05, 2018 – May 30, 2019)

The bulbs, stored during the summer season, were re-transferred into pots on September 05 and cultivated until the end of vegetation period. As shown in Table 3, some morphologic measurements, each one represented 10 individuals, were taken at the end of

the second cultivation year. The size of the pot-grown plants varied with bulb largeness and the photograph of mean-size individual representing plant growth and development in the second year is shown in Table 3. It was observed that basal leaf number per plant was 6.52, bulb diameter was 3.77 cm, scale number per bulb was 11.97, and total fresh biomass 53.80 g as mean values at the end of the second cultivation season. Total fresh biomass and scale number per bulb doubled when compared to the first-year data. But no individual blossomed at the end of the second year and for that reason, plant height could not be measured.

Table 2. Data for the plants with three or four basal leaves in first growing season.

Çizelge 2. Birinci vejetasyon yılını üç veya dört bazal yaprakla tamamlayan bitkilere ait veriler

| | Mean | SE | SD | Min. | Max. | CI (95%) |
|---|-------|------|------|-------|-------|---------------------|
|  Basal leaf fresh weight (g) | 0.91 | 0.02 | 0.07 | 0.80 | 1.02 | 0.855 ≤...≤ 0.957 |
| Basal leaf length (cm) | 7.79 | 0.28 | 0.88 | 6.19 | 8.89 | 7.162 ≤...≤ 8.416 |
| Basal leaf width (cm) | 2.58 | 0.06 | 0.19 | 2.30 | 2.90 | 2.444 ≤...≤ 2.722 |
| Root length (cm) | 7.48 | 0.20 | 0.62 | 6.61 | 8.61 | 7.034 ≤...≤ 7.922 |
| Root weight (g) | 1.73 | 0.05 | 0.16 | 1.42 | 1.93 | 1.620 ≤...≤ 1.848 |
| Bulb weight (g) | 5.87 | 0.22 | 0.69 | 4.61 | 6.73 | 5.376 ≤...≤ 6.366 |
| Bulb diameter (cm) | 2.61 | 0.07 | 0.22 | 2.20 | 2.94 | 2.448 ≤...≤ 2.767 |
| Bulb circumference (cm) | 7.79 | 0.25 | 0.79 | 6.87 | 9.12 | 7.226 ≤...≤ 8.358 |
| Scale number per bulb | 5.59 | 0.14 | 0.43 | 5.00 | 6.31 | 5.280 ≤...≤ 5.901 |
| Total fresh biomass (g) | 9.19 | 0.29 | 0.90 | 7.63 | 10.56 | 8.545 ≤...≤ 9.837 |
|  Basal leaf fresh weight (g) | 1.12 | 0.03 | 0.09 | 0.99 | 1.25 | 1.053 ≤...≤ 1.179 |
| Basal leaf length (cm) | 8.78 | 0.31 | 0.99 | 6.97 | 10.02 | 8.068 ≤...≤ 9.482 |
| Basal leaf width (cm) | 2.47 | 0.06 | 0.19 | 2.20 | 2.78 | 2.338 ≤...≤ 2.604 |
| Root length (cm) | 12.48 | 0.31 | 0.99 | 11.06 | 14.00 | 11.778 ≤...≤ 13.190 |
| Root weight (g) | 3.41 | 0.10 | 0.31 | 2.79 | 3.80 | 3.184 ≤...≤ 3.631 |
| Bulb weight (g) | 7.98 | 0.30 | 0.94 | 6.26 | 9.15 | 7.309 ≤...≤ 8.655 |
| Bulb diameter (cm) | 3.44 | 0.09 | 0.29 | 2.90 | 3.87 | 3.227 ≤...≤ 3.648 |
| Bulb circumference (cm) | 7.59 | 0.24 | 0.77 | 6.69 | 8.89 | 7.041 ≤...≤ 8.144 |
| Scale number per bulb | 7.83 | 0.19 | 0.61 | 7.00 | 8.84 | 7.392 ≤...≤ 8.261 |
| Total fresh biomass (g) | 11.04 | 0.34 | 1.09 | 9.17 | 12.69 | 10.266 ≤...≤ 11.818 |

*SE: Standart error, SD: Standart deviation, CI: Confidence interval

Growth performance of the bulbs in the third year (September 01, 2019 – May 30, 2020)

Bulbs of the second year were stored between June 05 - September 01 and planted in pots on September 02, 2019. They were cultivated during the third growing season and some measurements were taken as shown in Table 4. It was observed that mean values for root

and bulb weights were 29.8 and 47.6 g, respectively indicating plant growth exceeded double when compared to that of the second year. Together with the leaves formed on stem, mean value of leaf number per plant was 63. Scale number per bulb was also more than twice the previous year. But the most important phenomenon was that plants could reach

maturity and blossom. The bulb, root and flower of a third-year plant are shown in Table 4.

In the experimental region, pollination and insemination are insufficient due to the misty and humid climates. Thus, seed formation is weak and wild lily populations are also under pressure because of bulb harvesting for commercial purposes (Arslan et al., 2019). This phenomenon has forced to development of different economic and ecological production techniques. Generally, previous studies in this context provided similar results. Ozen *et al.* (2012) reported that wild *L. candidum* plants produced 2-12 basal leaves per plant dimensions of

which varied with 1.5-5 x 14-35 cm but these leaves became lost completely at flowering. As mean values, the width and height of *L. candidum* basal leaf were 3.4 and 19.7 cm, respectively. Our data in the present study did not confirm those of the former authors as we observed 1-4 basal leaves per plant, dimensions of which varied with 2.58 x 7.10-8.78 cm in the first year of cultivation and 5.79-7.33 basal leaves per plant in the second year. The difference between present and previous results could be attributed to growing media of plants because we cultivated experimental *L. candidum* plants in pots, but Ozen *et al.* (2012) used wild-growing plant material.

Table 3. Data for *L. candidum* plants from second year of pot cultivation

Çizelge 3. İkinci vejetasyon yılı sonu itibariyle *Lilium candidum* L. bitkilerine ait veriler

| | Mean | SE | SD | Min. | Max. | CI (95%) |
|-----------------------------|-------|------|------|-------|-------|---------------------|
| Basal leaf fresh weight (g) | 3.09 | 0.08 | 0.24 | 2.74 | 3.47 | 2.918 ≤...≤ 3.268 |
| Basal leaf number per plant | 6.52 | 0.15 | 0.49 | 5.79 | 7.33 | 6.175 ≤...≤ 6.873 |
| Root length (cm) | 30.30 | 0.85 | 2.69 | 25.77 | 34.33 | 28.377 ≤...≤ 32.222 |
| Root weight (g) | 6.95 | 0.16 | 0.49 | 6.04 | 7.64 | 6.598 ≤...≤ 7.305 |
| Bulb weight (g) | 14.65 | 0.37 | 1.16 | 12.98 | 16.42 | 13.821 ≤...≤ 15.478 |
| Bulb diameter (cm) | 3.77 | 0.08 | 0.27 | 3.28 | 4.15 | 3.579 ≤...≤ 3.963 |
| Bulb circumference (cm) | 10.70 | 0.24 | 0.76 | 9.30 | 11.77 | 10.158 ≤...≤ 11.247 |
| Scale number per bulb | 11.97 | 0.34 | 1.06 | 10.18 | 13.57 | 11.214 ≤...≤ 12.734 |
| Total fresh biomass (g) | 23.80 | 0.67 | 2.11 | 20.24 | 26.97 | 22.292 ≤...≤ 25.313 |

*SE: Standart error, SD: Standart deviation, CI: Confidence interval

Table 4. Data for *L. candidum* plants from third year of pot cultivation

Çizelge 4. Üçüncü vejetasyon yılı sonu itibariyle *Lilium candidum* L. bitkilerine ait veriler

| | Mean | SE | SD | Min. | Max. | CI (95%) |
|-------------------------|-------|-----|------|-------|-------|-------------------|
| Plant height (cm) | 84.0 | 4.3 | 13.6 | 67.0 | 102.7 | 74.3 ≤...≤ 93.7 |
| Leaf number per stem | 63.0 | 4.2 | 13.2 | 47.4 | 80.3 | 53.6 ≤...≤ 72.4 |
| Stem weight (g) | 98.8 | 5.8 | 18.2 | 76.6 | 123.3 | 85.8 ≤...≤ 111.8 |
| Stem diameter (cm) | 1.2 | 0.1 | 0.3 | 0.8 | 1.5 | 1.0 ≤...≤ 1.3 |
| Root length (cm) | 33.0 | 1.2 | 3.7 | 27.9 | 38.5 | 30.4 ≤...≤ 35.6 |
| Root weight (g) | 29.8 | 1.4 | 4.6 | 24.0 | 36.2 | 26.5 ≤...≤ 33.1 |
| Bulb weight (g) | 47.6 | 2.2 | 7.0 | 38.5 | 57.5 | 42.5 ≤...≤ 52.6 |
| Bulb diameter (cm) | 5.7 | 0.3 | 1.0 | 4.5 | 7.0 | 5.0 ≤...≤ 6.3 |
| Bulb circumference (cm) | 17.0 | 0.9 | 2.8 | 13.5 | 20.9 | 15.0 ≤...≤ 19.0 |
| Scale number per bulb | 28.5 | 1.3 | 4.0 | 23.3 | 34.2 | 25.6 ≤...≤ 31.4 |
| Total fresh biomass (g) | 176.2 | 9.4 | 29.8 | 139.1 | 216.9 | 154.8 ≤...≤ 197.5 |

*SE: Standart error, SD: Standart deviation, CI: Confidence interval

The leaves that dried completely at flowering were smaller than basal leaves and have become smaller from bottom to top on stems (Ozen et al., 2012). In the current study, leaf number per plant could be

measured in the third year and was found to be 47.4-80.3. Besides, plant height and stem width could also be measured in third year. Mean values were found to be 67.0-102.2 cm for plant height and 0.8-1.5 cm for

stem width. In concomitant with our results, plant height was reported as 43-150 cm (Ozen et al., 2012) and 105.3- 120.4 cm (Ozel and Erden, 2010). Ucar and Kazaz (2015) detected plant height as 26.4-74.7 cm and stem width as 5.0-9.5 mm. Kahraman (2015) used *L. candidum* bulbs, 4 cm in diameter as propagation material and reported plant height and stem width as 38.07-39.50 cm and 7.03-7.33 cm, respectively. The same author also searched the effects of different solid culturing media on some plant characters of greenhouse-grown *L. candidum* and reported plant height as 47.63-64.64 cm and stem width as 0.64-0.75 cm (Kahraman, 2014). In another previous study, conducted between 2012-2014 in seven locations, plant height and stem width were measured as 104.3-148.6 cm and 10.37-13.92 mm, respectively (Arslan et al., 2019). Herlina and Winarto (2019) detected plant height as 30-220 cm and stem width as 3.5-13.0 mm depending on main bulb size. The same authors reported that main bulb size affected positively plant height; the observed morphologic variation among *L. candidum* plants were caused by bulb age as well as species; and increasing bulb dimensions resulted in increasing mean values of plant characters.

Root weight (g) and length (cm) of the plants produced by stem derived bulbs were measured to screen root growth and development in the present study. *L. candidum* has yellowish-white hairy roots getting longer than 45 cm (Ozen et al., 2012). The root

length was reported to vary with 12.19-22.51 and 15.73-33.78 cm depending on the sampled wild populations and different growing media (Arslan et al., 2019). Eken and Sirin (2018) measured root weight for lily species as 3.590 - 11.037 g. Our results are accordant with those of the previous authors.

L. candidum has yellowish-white bulbs, 1.2-7.6 x 0.8-2.0 cm in dimensions and the bulbs bear approximately 50 scales (Ozen et al., 2012). In the present study, scale number per bulb was 3.00-8.84 depending on basal leaf number per plant in the first year and this value was recorded as 10.18-13.57 and 23.3-34.2 in second and third years, respectively, as expected (Figure 2). As mentioned in previous reports, main bulb size has affected significantly plant growth and development. We observed that our experimental plants could blossom hardly in the third year and bulb weight exhibited a great variation between 1.01 and 57.5 g during experiment (Figure 2). In previous papers, higher values such as 107.80-186.27 g (Ozel and Erden 2010), 97.33-393.19 g (Arslan et al., 2019) and 70.92-79.29 g (Kahraman, 2015) for bulb weight were reported. In contrast, Erken and Sirin (2018) reported lower values for the parameters (2.08-4.50 g). Kahraman (2015) observed an increase of 136.4-164.5% in bulb weight between main bulb and the harvested bulb at the end of one-year growing period. Similarly, we detected an increase of 226% in bulb weight between the second and third year bulbs (Table 3, 4; Figure 2).

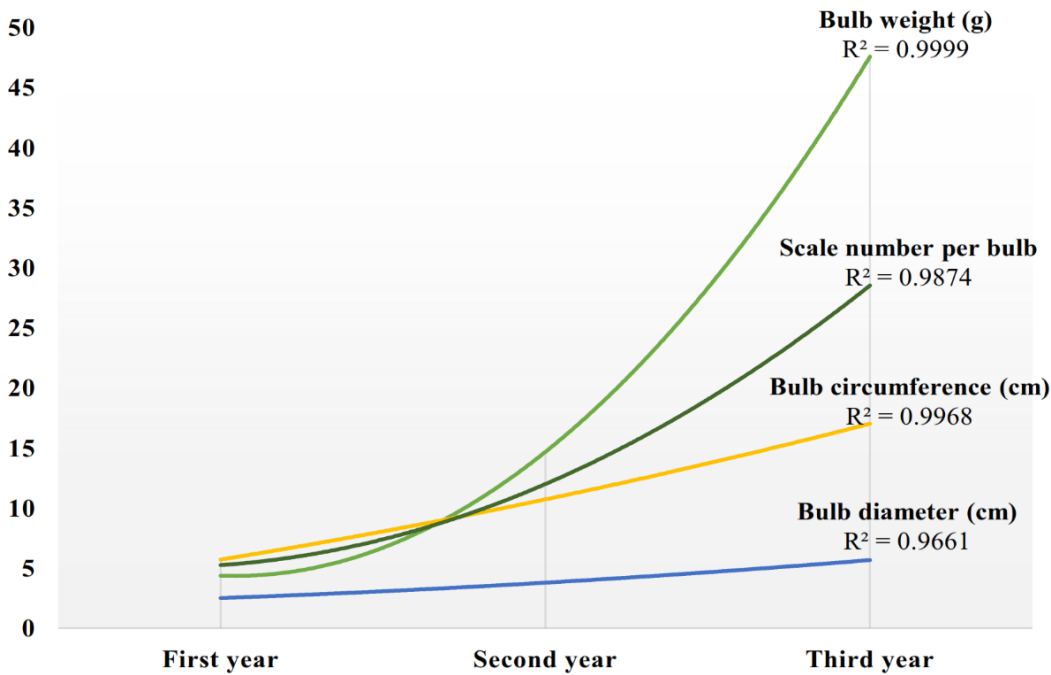


Figure 2. Variations in bulb characters of *L. candidum* during three years growth and development period.

Şekil 2. *Lilium candidum* L. bitkilerinin üç yıllık gelişme-büyüme döneminde soğan özelliklerinde meydana gelen değişime ait gösterim

Other parameters concerning bulb characters, evaluated in the current study are bulb diameter and

circumference. Our results for these parameters are greatly similar to the previous ones. As mean of three-year values, we determined bulb diameter as 1.4-7.0 cm (Figure 2) and this parameter was reported to be 0.8-2.0 cm (Ozen et al., 2012), 4.7-7.2 cm (Kahraman, 2014) and 5.92 cm (Kahraman, 2015). Similar to Kahraman (2015) who observed an increase of 48% in bulb diameter between main bulb and the harvested bulb, we also detected an increase of 51.2% in the same parameter (Figure 2). As for bulb circumference, this parameter exhibited the same change trend as bulb diameter and weight. Bulb circumference increased significantly at the rate of 58.9% when mean values of second and third years were compared and varied with 3.1 and 20.9 cm (Figure 2). Uysal and Kaya (2013) (5.6-6.4 cm), Ozel and Erden (2010) (23.6-28.3 cm) and Arslan et al., (2019) (16.7-35.6 cm) also reported similar results.

CONCLUSION

A number of studies have been conducted out so far on Madonna lily (*L. candidum*) propagation. In the previous efforts, generative or vegetative propagation methods via seeds and bulb scales and several in vitro techniques were tested. But two main obstacles were indicated in these previous reports. First, flower production is interrupted because fertile bulbs are needed as source of scale in the vegetative propagation of lilies via bulb scales. Second, seed-derived plants can reach sexual maturity hardly at the end of five years in generative propagation via seeds. When sufficient bulblets induction on stems were provided as described in the present study, a sustainable flower production can be continued without needing bulbs by re-using the stems of harvested plants as propagation material. In this study, we report for the first time that lily stems could produce 14 bulbs per stem on average and can be used effectively as source of bulb to use as propagation material. Considering these findings as well as the stem-derived bulbs that could transform into reproductive plants within three years, it is obvious that our results have made a significant contribution to the current knowledge on the propagation of Madonna lily that is under protection in accordance with CITES.

Researchers Contribution Rate Declaration Summary

Authors declares the contribution of the authors is equal.

Conflicts of Interest Statement

Authors have declared no conflict of interest.

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