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DIFFERENT APPROACHES ON BULBLET FORMATION WITH SCALING IN MADONNA LILY *(LILIUM CANDIDUM)*

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Abstract

The purpose of this study was, to determine the effects of different treatments on bulblet formation with scale propagation in 'Madonna lily' (Lilium candidum). The research was conducted in growth chamber at Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Horticulture in 2015-2016. Scales of Lilium candidum bulbs with 22-24 cm circumference, were used as a plant material. Effects of different incubation periods (10,12,14 weeks), incubation temperatures (10-15 °C, 20 -25°C), auxin (IBA 100 ppm, IBA 200 ppm) doses and scale positions (outer, middle, inner, center) on bulblet formation were investigated. The experiment was established according to randomised plot design with 3 replications. Some parameters like, bulblet formation ratio, bulblet number per scale, weight, diameter and height of bulblet, scale number, root number and root length of bulblet were also determined. As a conclusion, treatments have not any significant effect on bulblet formation ratio, but there were significant differences between the other parameters for some of treatments. In spite of this, incubation period of 14 weeks gives the highest average value for bulblet number per scale (1.467 piece) and bulblet height (19.105 mm). Also, the highest average value of bulblet weight (0.792 g) and bulblet diameter (13.282 mm) were measured in outer scales. While incubation temperature of 10-15 °C gives the best result for bulblet scale number (3.511 piece), the highest average value for root number of bulblet (3.900 piece) and root length of bulblet (11.224 cm) were measured in auxin dose of 200 ppm IBA.

Key words*: Lilium candidum, scale propagation, incubation, bulblet formation, ornamental plants*

INTRODUCTION

Turkey is very rich in terms of a plant diversity, in different three phytogeographic zones in the cross-point of Asia and Europe, along with the climatic change and soil properties. Turkey has about 12.000 taxa species and 3.750 of them are endemic (Avcı, 2005). Endemism ratio is 34.5 % (Uyanık et al., 2013). There are 1056 taxa geophytes and 424 of them are endemic (Özhatay, 2013). Geophytes, are the plants whose above ground parts such as stems, leaves and flowers dries and dies after completing their growth period and which in summer months live on thanks to their under earth storing parts such as bulbs, tubers and rhizomes, are also called natural flower bulbs. These are economically important in the sector of ornamental plants (Aksu et al., 2002; Zencirkıran, 2002). Some of these species have been exported over a hundred years. Bulb exportation of *Lilium candidum* are permited from only bulbs propagated in Turkey (Anonymous, 2016). *Lilium candidum* is globally known as "Madonna lily" or "white lily". It is a herbaceous, bulbous perennial plant belonging to *Liliaceae* family. It has fibrious roots and the roots are yellowish white colored. Its stem length is between 43-150 cm and has white flowers blooming between the end of May and the end of June, depending on the climate conditions (Özen et al., 2012). *L. candidum* can be propagated from seed and bulblets. However, propagation of seed takes five or more years from seed to develop plant capable of flower production. During the uprooting of the plant which takes such a long time to grow, newly sprouted, not ripened seedlings are also uprooted and therefore the damage increases more and more.

The objective of this study was, to develop some new methods with different approaches for scale propagation in *Lilium candidum* bulbs, which has been uprooted a lot from the nature and exported, also it was aimed on providing bulbs for exporting with these methods.

MATERIALS AND METHODS

This study has been conducted in 2015-2016 period in a growth chamber (Figure 1), where the temperature (^{o}C) and humidty (%) controlled by automatically, in COMU, Faculty of Agriculture, Department of Horticulture.

Figure 1. Inside of growth chamber

L. candidum bulbs with 22-24 cm in circumferences and 87.98 g in weight, were used as a plant material (Figure 2).

Figure 2. Bulb of *Lilium candidum*

The bulbs of *L. candidum* (Madonna lily) were provided from a firm exporting flower bulbs. The dry outer scales, any root remains and the bulb tip were all removed prior to scaling (Figure 3).

Scales were separated from basal plate of *L. candidum* bulbs (Figure 4), measured and calibrated for trials (Figure 5). Then washed with distilled water and sterilised with dilute alcohol.

Figure 3. *Lilium candidum* wihtout root

Figure 4. Scales separated from basal plate

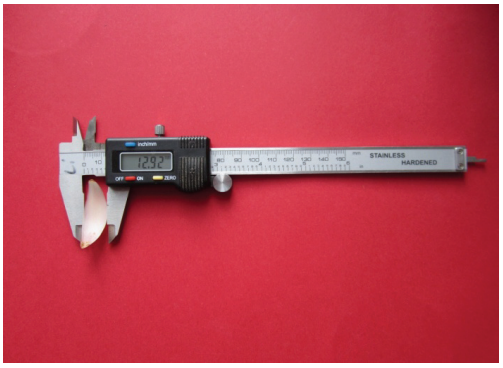

Figure 5. Calibration of lilium scales for trials

The average length of these scales is about 35.60 mm and the average width of the scales is 12.46 mm.

The scales were treated in 1% Captan and 0.5 % Mancozeb for 20 minutes to prevent fungal diseases (Figure 6), left in the shade for 10 minutes to remove the excess water, and kept in a cool place until planting.

Figure 6. Scales treated with fungicide

15 prepared bulb scales were mixed with 3 liters of damp perlite (Figure 7) and after the mixture was put to black polyethylene bags (5 L) (Figure 7). The bags were fastened tightly leaving some space on top (Figure 8,9), on October, 2nd 2015 (Aksu et al., 2002; Zencirkıran and Mengüç, 2002).

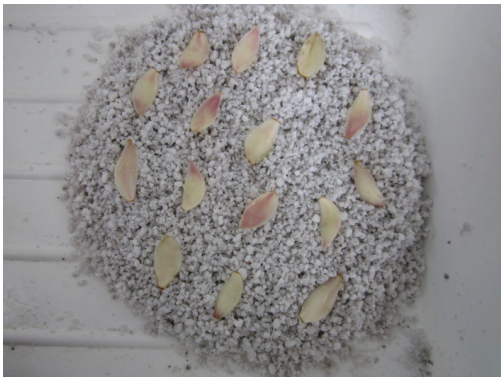

Figure 7. Perlite and scales

This research is consist of four different treatment such as incubation temperature, incubation period, scale position and auxin dose. In incubation temperature trial, the bags were incubated in a growth chamber at 10-15°C and 20-25 °C until January, 14th 2016 to form bulblets. Incubation period trial includes 10 weeks, 12 weeks and 14 weeks period. Scale position trial composed of outer, middle, inner, and center scales of Madonna lily. 100 ppm IBA, 200 IBA and control (without IBA) were used in auxin dose trial.

All experiment were established according to randomised plot design with 3 replications composed of 15 scales each.

Figure 8. Perlit and scale mixture inside of PE bag

After uprooting bulblets from bags, some parameters like bulblet formation ratio (%), bulblet number per scale (piece), bulblet weight (g), bulblet diameter (mm), bulblet height (mm), bulblet scale number (piece), root number of bulblet (piece) and root lenght of bulblet (cm) were measured.

The data were analyzed statistically by analysis of variance with SPSS 23. Separation of means was by the Duncan's multiple comparison test at $p = 0.05$.

Figure 9. Polyethylene bags in growth chamber

RESULTS AND DISCUSSIONS

According to Marinangeli and Curvetto (1997), bulblet formation or number of bulblets per scale were affected by some biotic factors such as cultivar, size, age, physiological status of the bulb, position of the scale in the bulb and by some abiotic factors such as, temperature, humidity, light, physiological and chemical treatments (Matsuo, 1987; Grassotti and Magnani, 1988; Magnani et al., 1988).

In this study it was determined that, incubation periods had a statistically significant effect ($p<0.05$) on only the bulblet number per scale. Maximum values of bulblet number per scale were obtained from 14 weeks of incubation period (1.467 piece).

On the other hand, there was not any significant difference between the three incubation period (Figure 10) (10,12,14 weeks) for the other bulblet characteristics (Table 1).

Figure 10. Scales and bulblets in incubation periodes

Table 1. The effect of incubation period on bulblet characteristics

Incubation Period	Bulblet Formation Ratio (%)	Bulblet Number per Scale (piece)	Bulblet Weight (g)	Bulblet Diameter (mm)	Bulblet Height (mm)	Bulblet Scale Number (piece)	Root Number of Bulblet (piece)	Root Lenght of Bulblet (cm)
10 Weeks	100.000 a	1.194 b	0.533 a	9.977 a	17.731 a	3.000 a	1.833 a	8.081 a
12 Weeks	96.970 a	1.067 b	0.629 a	10.912 a	18.158 a	2.938 a	1.806 a	9.483 a
14 Weeks	75.000 a	1.467 a	0.702 a	10.659 a	19.105 a	2.705 a	2.091 a	7.727 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$).

Many researchers stated that bulblet formation was also influenced by various plant growth regulators (Matsuo, 1972; Roh, 1990). In this research, similar results have been observed for auxin doses on some bulblet characteristics. Auxin doses had a statistically significant effect ($p<0.05$) on diameter and height of bulblet, bulblet scale number, root number and root length of bulblet (Figure 11).

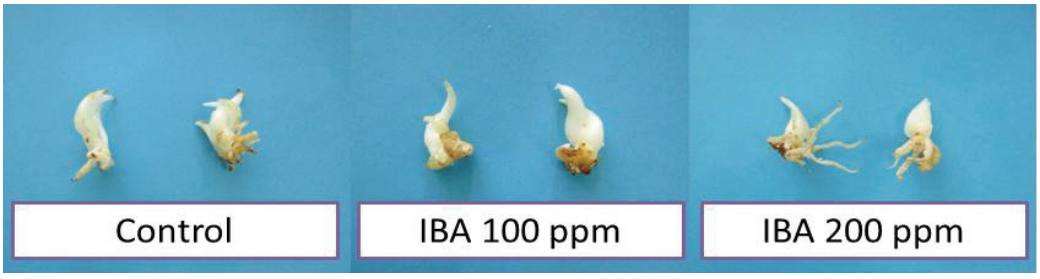

Figure 11. Bulblets in auxin doses and control

The highest value of bulblet diameter was 6.872 mm with 100 ppm IBA, while the lowest value of diameter was 5.446 mm with 200 ppm IBA (Table 2). Park (1996) reported that, generally the diameter of the bulblets was increased when the scales were treated with the 100 ppm IBA. With a value of 16.127 mm and 15.022 mm, 100 ppm IBA and control bulblets gives the best result for bulblet height. At the same time, the highest value was measured for bulblet scale number, at 100 ppm IBA (3.000 piece) and 200 ppm IBA (2.840 piece), respectively (Table 2).

Table 2. The effect of auxin doses on bulblet characteristics

Auxin Dose	Bulblet Formation Ratio (%)	Bulblet Number per Scale (piece)	Bulblet Weight (g)	Bulblet Diameter (mm)	Bulblet Height (mm)	Bulblet Scale Number (piece)	Root Number of Bulblet (piece)	Root Lenght of Bulblet (cm)
Control	22.932 a	1.083 a	0.268 a	6.398 ab	15.052 a	2.167 b	2.375 b	1.833 b
IBA 100 ppm	66.667 a	1.280 a	0.362 a	6.872 a	16.127 a	3.000 a	2.708 ab	9.152 a
IBA 200 ppm	48.889 a	1.300 a	0.249 a	5.446 b	11.356 b	2.840 a	3.900 a	11.224 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$)

Also, the highest value for root number of bulblets determined (3.900 piece) in 200 ppm IBA, similarly root length increased with auxin doses, thus, 200 ppm IBA gives the best result with an average value of 11.224 cm (Table 2).
Hence, growing structure of scales depends on their positions; it's an important factor for bulblet formation and characteristics. According to some researchers, scales from the outer and middle scales of lily bulb tended to produce more bulblets, which can be correlated with the total carbohydrate content in those scales (Matsuo, 1975; Park, 1996). Except bulblet formation ratio, all parameters affected significantly ($p<0.05$) by scale positions (Table 3). With 1.167 piece, the highest average value for bulblet number per scale was obtained from outer scales, other positions statistically acted at same level on bulblet number per scale. For weight, diameter, height, scale number and root number of bulblets, similar results observed in scale positions (Figure 12).

Table 3. The effect of scale positions on bulblet characteristics

Scale Position	Bulblet Formation Ratio (%)	Bulblet Number per Scale (piece)	Bulblet Weight (g)	Bulblet Diameter (mm)	Bulblet Height (mm)	Bulblet Scale Number (piece)	Root Number of Bulblet (piece)	Root Lenght of Bulblet (cm)
Outer Scale	83.335 a	1.167 a	0.792 a	13.282 a	18.319 a	3.500 a	2.273 ab	3.955 b
Middle Scale	80.000 a	1.028 b	0.517 b	9.473 b	15.824 b	2.765 b	2.355 a	10.468 a
Inner Scale	90.790 a	1.025 b	0.462 b	8.788 b	14.207 b	2.769 b	1.897 bc	10.000 a
Center Scale	79.840 a	1.000 b	0.236 c	7.107 c	12.036 c	2.000 c	1.500 c	8.813 a

Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test ($p< 0.05$).

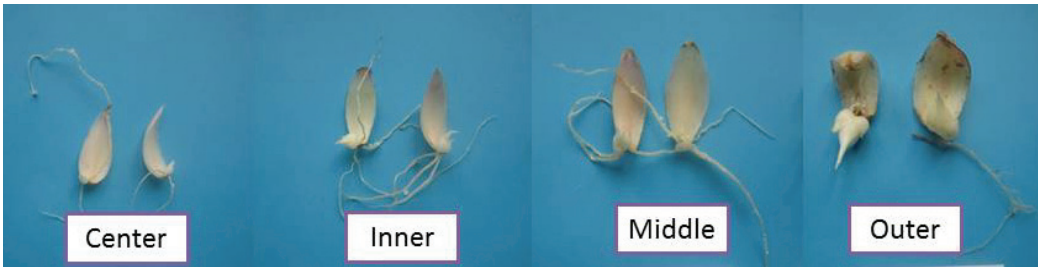

Figure 12. Scales and bulblets in scale positions

When the highest values measured in outer scales, center scales were give the lowest values, generally middle and inner scales statistically acted at the same level. So it was

clear that, there was an increase from center to outer scales. On the other hand, it was not investigated for root length of bulblet (Table 3). According to the results of Matsuo et al. (1987), larger scales produce more bulblets in Lily. Hanks (1985) reported that, the number and sizes of differentiated bulblets are influenced by the relative position of starting scales in Narcissus bulbs. Marinangeli et al. (2003) conclude that, middle scales are the best starting materials for experimental uses involving scale propagation and external scales must be included for production. Also, Padasht et al. (2006) reported that, outer and middle scales at 20 and 25 °C regenerated more bulblets with better properties than inner scales. Many researcher reported that, the growth and development properties of newly formed bulblets depends on the temperature during scaling (Van Tuyl, 1983; Aquettaz et al., 1990). Our results for *L. candidum* indicates that incubation temperatures had a statistically significant effect ($p<0.05$) on only the diameter and root length of bulblet (Table 4) (Figure 13).

Table 4. The effect of incubation temperature on bulblet characteristics

Incubation Temperature	Bulblet Formation Ratio (%)	Bulblet Number per Scale (piece)	Bulblet Weight (g)	Bulblet Diameter (mm)	Bulblet Height (mm)	Bulblet Scale Number (piece)	Root Number of Bulblet (piece)	Root Lenght of Bulblet (cm)
10-15 °C	95.553 a	1.089 a	0.410 a	7.933 b	17.279 a	3.511 a	2.178 a	5.658 b
20-25 °C	100.000 a	1.133 a	0.427 a	9.056 a	16.378 a	2.889 a	2.133 a	7.351 a
	ns	ns	ns	*	ns	**	ns	*

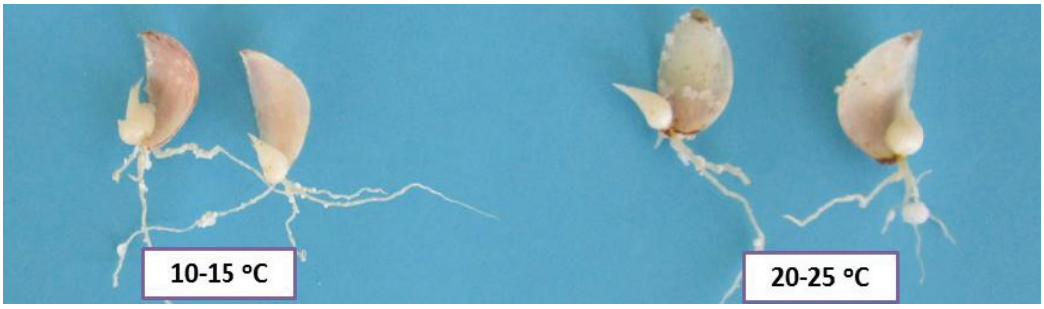

Figure 13. Scales and bulblets in incubation temperatures

The highest value for diameter of bulblet (9.056 mm) and root length of bulblet (7.351 cm) were taken from incubation temperature of 20-25 °C (Table 4). Similar results had been observed by Suh and Lee (2006), the number or bulblets produced per scale in both lilium varieties used in the trial, were not affected by incubation temperatures, however the diameter of 'Casablanca' bulblets were icreased as temperatures was increased from 20 °C to 30 °C. On the other hand, treatments have not any statistically significant effect on bulblet formation ratio, but there were only some quantitative differences between the treatments (Figure 14).

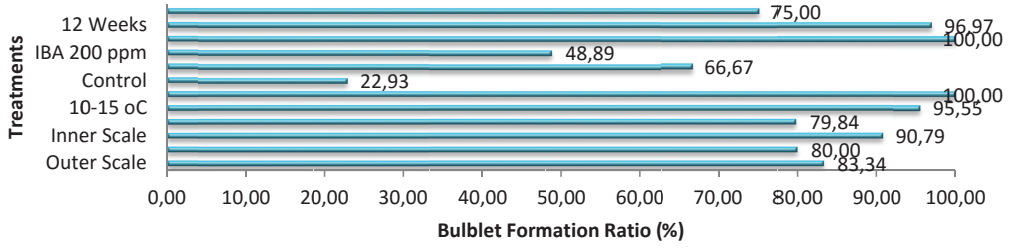

Figure 14. Changes on bulblet formation according to treatments.

The correlation coefficients related in bulblet characteristics are given in Table 5. According to this results, the strongest relationship (r=0.841) in *L. candidum* was found between the bulb diameter and bulb weight. In spite of this, there was a positive correlation between bulblet height (r=0.770) and bulblet weight and also between bulblet height (r=0.639) and bulblet diameter, while there was a negatively weak relationship (r=-0.189) between root length of bulblet and bulblet number per scale (Table 5).

Table 5. Correlation between the bulblet characteristics of *Lilium candidum*

	Bulblet Formation Ratio	Bulblet Number per Scale	Bulblet Weight	Bulblet Diameter	Bulblet Height	Bulblet Scale Number	Root Number of Bulblet	Root Lenght of Bulblet
Bulblet Formation Ratio	1							
Bulblet Number per Scale	-,008	1						
Bulblet Weight	,306	,100	1					
Bulblet Diameter	,247	,138	**,841****	1				
Bulblet Height	-,092	,108	**,770****	**,639****	1			
Bulblet Scale Number	,500	-,028	**,631****	**,609****	**,595****	1		
Root Number of Bulblet	,060	,016	**,448****	**,436****	**,291****	**,462****	1	
Root Lenght of Bulblet	,233	**-,189***	,180	,093	,040	,162	**,364****	1

*. Correlation is significant at the 0.05 level
**. Correlation is significant at the 0.01 level

Consequently, there were some positive relations too between bulblet scale number with bulblet weight (r=0.631), bulblet diameter (r=0.609) and bulblet height (r=0.595) respectively. Similar relations were found between root number of bulblet with bulblet weight (r=0.448), bulblet diameter (r=0.436), bulblet height (r=0.291) and also with bulblet scale number (r=0.462) respectively. It is seen on Table 5 that, there was an another relation observed between root length of bulblet with root number of bulblet (r=0.364).

CONCLUSIONS

This research was focused on the effects of different treatments, such as incubation period, incubation temperature, auxin doses and scale position on bulblet formation and characteristics with scale propagation in 'Madonna Lily' (*Lilium candidum*).

The overall results indicate that, treatments have not any significant effect on bulblet formation ratio, but there were significant differences between the other bulblet characteristics for some of treatments. Especially for the incubation period, 14 weeks gives the highest average value for bulblet number per scale and bulblet height. However, study results shows that 10 week is sufficient for an incubation period. We conclude that, the highest average value of bulblet weight and bulblet diameter were determined in outer scales. So, it was cleared that outer scales are the best propagating material, in addition to, middle scales could be used for propagation. While incubation temperature of 10-15 ^{0}C gives the highest value for bulblet scale number, 20-25 ^{o}C was more effective for bulblet diameter and root length of bulblet. Also, importance of auxins on rooting factors were understood once again with results of the study. As a conclusion it was seen that, different approaches and proper methods were necessary for scaling procedure in *L. candidum*. However, for geophytes there was not any specific information about formation, growth and development of new bulblets.

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