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Extending Storage Duration of Mother Scales for Enlarging Scale Bulblets and Soluble Carbohydrates Content in Lily "Arabian Red"

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Abstract

Oriental hybrid lily "Arabian Red" mother scales were stored in moist cocopeat and perlite (1:1) for 3 or 5 months at 25 ˚C. The numbers of scale bulblets per mother scale were nearly identical in two durations. Increasing storage duration promoted the growth of roots (number and length) and No. scales per scale bulblets. And, diameters of scale bulblets were more in longer storage duration (5 months) than 3 months. Therefore, storage duration affects size, no quantity of scale bulblets in "Arabian Red" lily. This can cause with giving opportunity to scale bulblet for more consuming the reserves of parent scale and extending the root growth, then produce greater bulblet. The results show that scale bulblets had more sucrose and glucose in 5 months, the content of fructose did not change in development process nevertheless, it seems, that has not any effect on the sprouting bulblets.

Keywords: Asexual propagation, *Lilium*, Scale bulblet, Scaling, Sugars.

INTRODUCTION

In addition to *in vitro* culture, other asexual methods for propagation of *Lilium longiflorum* and other hybrids including orinetal, asiatic, LA etc. are scaling or by stem bulblets. To produce the forcing-sized bulbs from scale or stem bulblets are required three or two growing seasons, respectively (Kim *et al.*, 2007). The scale of lily bulb is a kind of transformed leaf and has only a thin epidermis (Matsuo *et al*., 1989). Scaling is a technique for *Lilium* propagation (Gray, 1974; Marinangeli and Curvetto, 1997; Marinangeli *et al*., 2003). Scales are separated from the basal plate of mother bulb and placed in a favorable environment. Adventitious bulblets form on the end of individual bulb scales, regardless of the orientation of the scale when planted (Gray, 1974).

This technique was reported and studied previously (Matsuo, 1972; Matsuo and Van Tuyl, 1984; Matsuo *et al.*, 1989; Cabrera, 2002). External and middle scales have more weight than internal scales, and produce more scale bulblets. The scale bulblets induce at different positions (external, middle and internal scales) in any cultivar. In "Chotaro", more bulblets were produced from middle and internal scales than external scales. However, in "Hinomoto" and "American White" were the reverse (Matsuo *et al*., 1989). Matsuo *et al.* (1989) demonstrated that the reason was that internal and middle scales greater than external scales in "Chotaro".

The sucrose is an essential and major carbohydrate in *Lilium* bulb, because breaking and metabolism of this sugar are important to facilitate the growth of storage organ, inflorescence and others (Addai, 2010). However, Addai (2010) noticed that starch is a major carbohydrate and energy source for bulbous plants such as lily before vernalization. After vernalization, starch breaks to sucrose and reducing sugars (Xu *et al*., 2006), this phenomenon referred to low temperature sweetening, and has been reported in *Lilium*, *Hyacinthus* and *Tulipa* as well (Miller and Langhans 1990).

The effects of scale position, size and storage duration on number of bulblets (no the diameter or size of induced bulblet) were reported in "Stargazer" (Park, 1996). The two research goals were: 1) determine how storage duration affects on the yield of scale bulblets. 2) determine the physiological effects of storage duration on soluble carbohydrates. Because, the content of carbohydrate affects on quality of sprouting scale bulblets after vernalization.

MATERIALS AND METHODS

Plant material

Oriental hybrid lily "Arabian Red" bulbs (18-20 cm in circumference, Van den Bos, Netherlands) were scaled. Middle scales on basal plate with same weight (2-2.5 g) were used in this experiment. Scales were dipped for 15 min in a fungicide solution containing 1% (w/v) Benomyl. After air drying, 10 scales were planted in polyethylene bags with some punctures containing ≈ 1 liter of moist cocopeat and perlite (1:1, v:v; 85-90% moist). The bags were placed at 25 ˚C in the growth chamber for 3 or 5 months (Marinangeli and Curvetto, 1997; Fig. 1). After storage duration (3 or 5 months) were recorded number of scale bulblets per scale, number of main roots, lengths of main root (cm), diameter of scale bulblet (mm, Digital caliper, Japan, accuracy: ± 0.02) and number of scales per scale bulblet. The soluble carbohydrates in the vernalized scale bulblets (2 months at 3-5 ˚C) were analyzed with following method.

Fig. 1. The storage of scales at polyethylene bags containing cocopeat and perlite (1:1) in 25 ˚C to induce and produce scale bullets.

Soluble carbohydrates

1. Materials. Ethanol, glucose, fructose, sucrose with analytical grade and sodium citrate buffer and acetonitrile with HPLC grade were purchased (Merk, Germany).

2. Extraction. To extract the soluble carbohydrates, scale tissues of bulblets were powdered, and 0.5 g DW (freeze drying) was put in a test tube. Then, 10 ml ethanol 80% was added and homogenized completely. Homogenized solution was replaced in centrifuge tube and centrifuge in 8000 × at 15 min. Supernatant was filtered with 0.2 µm filters and injected to HPLC (Shin *et al.*, 2002).

3. Assay. HPLC (High Performance Liquid Chromatography; UNICAM-Crystal-200; UK) was used to assay and measurement of soluble carbohydrates (sucrose, glucose and fructose) in extracted samples from scale bulblets. This investment had a gradient pump with column 80 Å and UV detector in 320 nm and injector (Model 7125, Rheodyne, Cotati, USA) 20 µl. Mobile phases were sodium citrate buffer (pH = 5.5) and acetonitrile (1:99), and were with rate 0.1 mm/min. According to stop time and standards, kind and amount of sugars in unknown samples were identified (Gomis *et al.*, 2001).

Experimental design and treatments

The experimental design was a randomized completely design with 2 storage durations (3 or 5 months) in 7 replications for non-destructive and 3 replications for destructive characters (soluble carbohydrates). Data were subjected to analysis of variance using SAS (Statistical Analysis System, SAS Institute, Cary, NC). Means were separated HSD (Tukey) test.

RESULTS

Based on data analysis of variance, the effect of storage durations were significant on the scale bulblet diameter, number of scales per bulblet, length and number of main roots. However, between two storage durations (3 and 5 months) was not significant different on the bulblet number per mother scale (Table 1 and 2).

More and longer roots were grown in 5 months storage duration compared to 3 months (Table 2). The more scale were induced and developed per scale bulblet when mother scales were stored to 5 months (Table 2).

The storage of mother scales in 5 months caused to produce larger scale bulblets compared

Table 1. Analysis of variance for storage duration of mother scales on growth and development of yearling bulblets in Oriental lily "Arabian Red".

Source of variation	Mean of squares					
	df	Bulblet No. per mother scale	Main roots No.	Length of main roots (cm)[w]	Diameter of scale bulblet (mm)	Scale No. per scale bulblet[y]
Treatment	1	0.27 [ns]	2.51 **	789.88 **	0.62 *	4.44 **
Error	12	0.07	0.11	2.34	0.13	0.16
CV (%)		13.71	14.98	8.52	3.67	8.14

[ns], *, ** Treatment is not significant, or significant at 0.05≥ α >0.01 or at 0.01≥ α > 0.001, respectively.
[w] The length of longest root was measured.
[y] df of error of scale No. per scale bulblet is 8.

Table 2. Effects of storage duration (months) on growth and development of scale bulblets in Oriental lily "Arabian Red".

Storage duration (months)	Bulblet No. per mother scale	Main roots No.	Length of main roots (cm)	Diameter of scale bulblet (mm)	Scale No. per scale bulblet
3	2.07 ± 0.07 [a]	1.8 ± 0.08 [b]	10.42 ± 0.55 [b]	9.53 ± 0.16 [b]	4.27 ± 0.22 [b]
5	1.79 ± 0.12 [a]	2.65 ± 0.16 [a]	25.44 ± 0.60 [a]	9.95 ± 0.10 [a]	5.60 ± 0.12 [a]

Means in each column followed by similar letters are not significantly different at 5% according to HSD test. Each mean is ± SE for 7 plants (5 plants in scale No. per scale bulblet).

Table 3. Analysis of variance of mother scales storage duration effects on content of soluble carbohydrates at period of scale bulblets production.

Source of variations	df	MS		
		Sucrose	Glucose	Fructose
Treatment	1	3.79*	1.39*	2.007 ns
Error	4	0.37	0.14	0.42
CV%		15.31	10.20	20.96

ns no significant difference, * significant at p≤0.05, respectively.

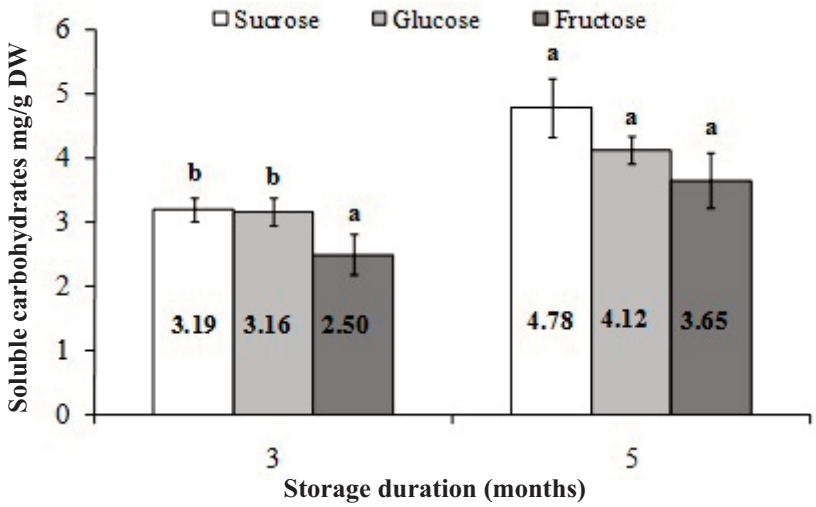

Fig. 2. Effects of storage duration (months) on soluble carbohydrates in vernalized scale bulblets in Oriental lily "Arabian Red". The same color columns with similar letters have not significant differences in HSD0.05. The bars are SE± 3.

to storage duration in 3 months. Furthermore, the number of scales per bulblet was more; however, the number of scale bulblets were not increased (Table 2).

The storage of mother scales in 5 months caused to produce larger scale bulblets compared to storage duration in 3 months. Furthermore, the number of scales per bulblet was more; however, the number of scale bulblets were not increased (Table 2).

The effects of storage durations (3 and 5 months) on the content of major soluble sugars (sucrose, glucose and fructose) in lily bulb tissue are shown in Table 3. According to the results, storage duration had significant effects (p≤0.05) on sucrose and glucose, but fructose has not been affected by storage duration. With increasing storage duration from 3 to 5 months, content of sucrose and glucose increased 49.84 and 30.38% respectively (Fig. 2).

The correlation coefficients between evaluated parameters in this work showed that scale bulblet diameter had the direct and significant correlation with main root number. The increasing main roots at storage period raised the content of fructose in vernalized scale bulblet. According to correlation coefficients, there are positive and significant effect between root length and content of sucrose and glucose. The content of sucrose and glucose show direct correlation (Table 4).

DISCUSSION

It seems, in "Arabian Red", increasing the storage duration doesn't affect on the producing more scale bulblets (Fig. 3). Park (1996) studied on the effects of storage durations (3 or 5 months) on yield of scale bulblets in *Lilium* "Stargazer", he reported that yield was increased with number (no size) of scale bulblet, which is contrary to this work. Furthermore, Matsuo and Van Tuyl (1984) reported the number of scale bulblets developed per mother scale were different with storage durations and scale positions. But Gray (1974) reported depending on the species and cultivar, each

Table 4. Correlation coefficients between growth and development of scale bulblet and soluble carbohydrates at period of scale bulblets production.

Parameters	SBD	MRN	LMR	SN	BN	Suc	Glu	Fru
SBD	1.00							
MRN	0.90*	1.00						
LMR	0.40	0.74	1.00					
SN	0.04	0.14	0.34	1.00				
BN	-0.30	-0.54	-0.59	-0.11	1.00			
Suc	0.17	0.50	0.85*	-0.02	-0.28	1.00		
Glu	0.07	0.43	0.85*	-0.03	-0.40	0.98**	1.00	
Fru	0.68	0.86*	0.76	-0.20	-0.64	0.72	0.71	1.00

**, * Correlation (two tailed) in P≤ 0.01 and 0.05 respectively, n= 6.
(SBD); Scale bublet diameter (mm), (MRN);No. main root, (LMR); length of main roots (cm), (SN); Scale No. per scale bulblet, (BN); Bulblet No. per mother scale, (Suc); Sucrose (mg/ g DW), (Glu); Glucose (mg/ g DW), (Fru); Fructose (mg/g DW)

scale will usually develop 3 to 5 bulblets.

Roots were produced and developed in the base of mother scales and scale bulblets (Fig. 4). The roots can be benefit to uptake water from substrate and to protect mother scale and scale bulblets and after growth of scale bulblets for producing yearling bulblets. The development of bulblets from mother scales of Olympic, Fiesta, and Bellingham hybrids contrary to *Lilium* longiflorum, vascular connections didn't arise between the mother scale and the bulblet primordia. In addition, the bulblet was dependent upon the parent scale during its early stages of initiation (Gray, 1974).

The increasing duration of storage to 5 months was increased diameter of scale bulblet 4.407% compared to 3 months. The size of bulblet affects the quick and uniform sprout and produce efficiently photosynthesis leaves and bulb enlarge rapidly (Langens-Gerrits *et al*., 2003). After scaling, and production of scale bulblets, three different types of plants grow from scale bulblets (Matsuo, 1972; Matsuo and Van Tuyl, 1984; Matsuo *et al.,* 1989). The scale bulblets that grow in the development of ETP (Epigeous type plant), enlarge rapidly to produce yearling bulblet and forcing sized bulb. These plants produce one foliated stem, other plant is hypogeous type plant (HTP) that has only scaly leaves, and other plants are hypo-epigeous type plants (HETP) that have stem and scaly leaves. Matsuo and Van Tuyl (1984) demonstrated that bulb storage temperature and duration affect on scale bulblet development and type of plant development. Han *et al.* (2005) reported the normal bulblets formed shoots with one foliated stem, and they grown normally in greenhouse, after cold treatment at 5 °C for 2 months. Most of the bulblets produced stems with several leaves. These bulblets grow rapidly in soil and they increase the frequency of large commercial bulbs in a short time. Fast growth occurred in large bulblets and in bulblets that formed a

Fig. 3. Scale bulblet at mother scale base of Oriental lily “Arabian Red” after 4 months in 25 ˚C.

Fig. 4. The growth of root during scale propagation in Oriental lily “Arabian Red” at cocopeat: prlite (1:1) substrate in 25 ˚C.

stem with several leaves instead of one or two leaf-bearing scales. Since stem formation occurred more often in large bulblets, *in vitro* bulblet growth is an important factor for rapid growth of lily bulblets after transplantation (Han *et al*., 2005).

It seems the increasing root number causes that scale bulblet diameter increase. Moreover, Increasing number and length of roots promote the content of sugars with extending storage duration (Table 4). As it was demonstrated with Gray (1974), "under ordinary conditions, root development was not observed until after the formation of several leaf primordia. In the early stages of growth, root and bud primordia are independent of one another. As development continues, however, the parenchyma between the root and bud primordia becomes meristematic, and forms cells which become differentiated into vascular elements connecting the primordia. At no time is there any indication of a vascular connection between the vascular elements of the root and bud primordia and the mother bulb scale". Therefore, by extending the duration of storage, while can cause the consumption of parent scale reserves with induced scale bulblet, furthermore, cause the development of roots and more growth of scale bulblet.

According to storage duration effects on carbohydrate content (Fig. 2) and as other researchers noticed (Xu *et al.*, 2006; Addia 2010), sucrose and glucose are major carbohydrates for sprouting bulblets and bulbs of lily after vernalization. In this experiment, content of fructose had not different with other 2 sugars. However, storage duration had no effect on its contents. Therefore, it can interpret that fructose has not any major effect on sprouting bulb and do not change in the development process. Miller and Langhans (1990) reported daughter scales of L. *longiflorum* bulb 'Nellie White', which vernalized at 4.5 °C in 60 days, approximately had 75 mg / g sucrose, 2 mg / g glucose and 2 mg / gfructose in their tissues. Moreover, Shin et al. (2002) vernalized *in vitro*-produced bulblets of oriental lily 'Casablanca' at 4 ˚C in 8 weeks. They observed that content of sucrose, glucose and fructose were 22, 8 and 1.8 mg /g FW, respectively. They also reported that the content of fructose in 8th week was similar to first day of vernalization of bulblets.

CONCLUSION

Longer storage duration caused increasing of yield as greater scale bulblets with more scale per bulblet. And, it was induced more and longer roots at bulblet in five months. Storing mother scales for longer time (5 months compared to 3 months) causes to produce scale bulblets with more quality. Sucrose and glucose showed 50% increasing in content approximately. Scale bulblets after five months storage had higher stored carbohydrates, therefore were high qualify and sprout earlier.

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