Effects of Morphological Characteristics, Nutritional Status and Light on the Scale Propagation of Lilium

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Abstract: Scaling is the most commonly used technique to artificially propagate lilies. Scales from different positions of the donor bulb vary in regeneration efficiency; however, the mechanism underlying bulblet formation remains unclear. To investigate the relationship between scale morphological characteristics, initial nutrient status and bulblet regeneration capacities during scale propagation of Lilium, we performed comprehensive morphological and correlation analyses using scales from three lily cultivars. Principal component analysis clearly distinguished middle scale (MS) from outer scale (OS) by morphological characteristics alone. Morphological results indicated that MS and OS differ significantly in terms of scale width, facial area, basal area, volume, length-to-width ratio and width-to-thickness ratio. Correlation analysis showed that scale width was significantly positively correlated with both the quantity and quality of regenerated bulblets. Among the cultivars, starch and soluble sugars accounted for 50–80% of scale DW. And a higher initial ratio of sucrose to starch in scales was more conducive to the bulblets formation. Although light had no effect on the incidence of bulblets, the formation of bulblets was positively enhanced, and better morphological consistency was obtained. This present study achieved a comprehensive morphological and nutritional analysis focused on bulblet formation capacities of scales from different positions of lily bulbs via scaling propagation, laying a foundation for future molecular studies on bulblet formation.

Keywords: bulblet formation; morphology; nonstructural carbohydrate; bulb production

1. Introduction

Asexual reproduction, the biological process of creating a new individual asexually through vegetative shoot meristems [1], is essential for the survival of plant species to overcome harsh environmental conditions [2,3]. Moreover, the clones originating from vegetative meristems are identical to the mother plant, which is a desired trait in agriculture and horticulture industries [4,5]. Lily (Lilium spp.), a monocotyledonous bulbous plant of the Liliaceae family, is one of the major ornamental geophytes in the global floriculture industry and has high ornamental, medicinal and edible value [6–8]. Most lily species are propagated by scaling, the most commonly used artificial vegetative propagation technique, to maintain genetic purity [9–12]. The results of previous studies on the effects of scaling in lily can be summarized in terms of two main aspects, that is, internal and external causes. Specifically, the influencing internal factors on scaling mainly include the tissue source and physiological status of scales, whereas the influencing of external factors mainly come from the incubation environment (e.g., temperature, humidity and light) and physiological and chemical treatments [13,14]. Although bulblets are widely produced from scaling for conventional commercial vegetative propagation, scaling is still one of the main research topics in the industrialization of lily bulbs.

Lily bulbs contain specialized scales (modified leaves) that serve as the primary storage tissue attached to the basal plate. Those scales have the remarkable capacity to regenerate...
new plantlets (bulblets) by initiating de novo shoot meristems from excised places without the addition of exogenous hormone regulators [5]. Several cellular and physiological studies have been performed in the past, aiming to investigate the possible factors that grant lily scales their competence to regenerate. Generally, outer and middle scales regenerate more bulblets with better properties than inner scales [9], which raises the question of whether differences in the morphological characteristics and physiological status of scales could influence bulblet regeneration capacity and how these features correlate.

Carbohydrates can serve as building blocks and as an energy source for the formation and development of the nonphotosynthetic apparatus in bulbous plants [15–17]. The whole scaling process of lily is actually a process of reducing starch to soluble sugar under the action of amylase in mother scales for the formation and development of regenerated bulblets [18]. Plant reproduction depends greatly on adequate import of photoassimilates, which, for most plant species, are mainly in the form of sucrose [19]. To date, studies on the changes in carbohydrate content and the expression of related genes during bulblet formation and development in flower bulbs have revealed strong regulation of starch and sucrose metabolism during this process [20–23]. However, details about the initial nonstructural carbohydrate landscape of scales from different positions of bulbs associated with the quantitative (number) and qualitative (weight) traits of bulblets are essentially unknown.

Appropriate environmental conditions can improve the differentiation ability and bulblet formation efficiency of scales. To date, some achievements have been made in the selection of substrates and the application of plant growth regulators. The effects of light on the bulblet regeneration of lily have been widely reported under in vitro conditions; however, little progress has been made in scaling research. Previous studies have shown that light can regulate the content and proportion of hormones in scales and promote the degradation and utilization of nutrients in scales. A moderate reduction in light intensity can improve the photosynthetic capacity of the scales, which is conducive to scale differentiation [24]. Although previous studies reported that light had a significant effect on the differentiation number and biomass accumulation of bulblets [24], how to better apply light treatment during scaling is still worth further research.

Scaling provides an excellent model to study bulblet regeneration in lily from single scales, which contain all the necessary components for the initiation of meristems and further development [25]. To understand the relationship between scale morphological characteristics, initial nutrient status and bulblet regeneration capacities during the scale propagation of Lilium, we performed detailed morphological and correlation analyses of scales from different positions of lily bulbs. By applying light treatments, we also compared the bulblet incidence rate and regeneration efficiency of different scales. This work provides comprehensive morphological and nutritional information on bulblet formation via scaling propagation, providing useful information for future molecular studies on bulblet formation.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Bulblet induction experiments were conducted at Cornell University, Ithaca, NY, USA. Dutch-grown bulbs of Lilium LA Hybrid ‘Nashville’ (NA), Lilium Oriental Hybrid ‘Sorbonne’ (SOR) and Lilium OT Hybrid ‘Yelloween’ (YE) were collected before flowering. Scales were carefully peeled from the fresh bulbs and surface sterilized with 1% captan for 30 min followed by three rinses with distilled water. Clean and healthy scales were selected, placed in plastic trays and cultured at 20 ± 2 °C in growth chambers with 60–70% humidity under light (100 µmol·m⁻²·s⁻¹, 12 h of photosynthetic photon flux density from cool-white fluorescent lamps) or dark (24 h) conditions. Scales were sprayed with distilled water two times per day to minimize desiccation.

2.2. Morphological Observation and Sampling

Morphological changes in scale propagation were carefully observed and recorded in the 3 days for the first 2 weeks (1 day after propagation (DAP), 4 DAP, 7 DAP, 9 DAP
and 14 DAP) and every week thereafter (21 DAP, 28 DAP, 35 DAP and 63 DAP). The length, width and thickness (Figure 1e,f) of the scales were measured using Vernier calipers, and the facial area (FA), basal area (BA) and volume of the scales were calculated as mentioned below. The widest part was consistently selected for recording. Scales were divided into two groups, outer scale (OS) and middle scale (MS), according to their physiological position in bulbs. Samples for soluble sugar and starch extraction were collected during scale propagation at 0 DAP and 63 DAP, respectively. The scale samples were flash frozen in nitrogen and stored at $-80^\circ$C until being freeze-dried.

Facial area = scale length × scale width,

Basal area = scale length × scale thickness,

Volume = scale length × scale width × scale thickness,

\[
\text{Regeneration rate} = \frac{\text{Number of scales that produced bulblets}}{\text{Total number of scales}} \times 100\%.
\]

Figure 1. Morphological characteristics of scales from three lily cultivars. (a–c) Morphological changing patterns of scales from outer layer to inner layer in three lily cultivars. (a) Scale width, (b) Scale length, (c) Scale thickness. The solid bars are biological replicate data (n = 10). (d) Morphological comparison of outer scales and middle scales. Bar = 1 cm, (e,f) represent the length, width and thickness of scales, respectively. (g) Principal component analysis (PCA) of morphological characteristics of scales from different position of three lily cultivars. NA, Nashville; SOR, Sorbonne; YE, Yelloween; OS, outer scale; MS, middle scale.

2.3. Extraction of Nonstructural Carbohydrates in Lily Scales

The freeze-dried samples were ground with a Wiley mill (mesh size 40) to a fine powder for nonstructural carbohydrate extraction [26,27] and stored at $-20^\circ$C with desiccant before use. Five scales were treated as an individual sample throughout the extraction and determination processes. Samples were examined using the modified ethanol-soluble method as described by Ranwala and Miller [27]. Tissue samples (50 mg dry tissue) were extracted with 80% ethanol at 70 °C, 3 mL each time for 30 min. Specifically, each conical
centrifuge tube containing tissue samples and extractant was vortexed for 5 s and placed in a water bath for 30 min of extraction. At the end of each extraction, each tube was vortexed again for 5 s before centrifugation (4000 × g, 10 min) and the supernatants were combined for a total of 9 mL. Lactose (100 µL) was added to each sample as the internal standard. The water–alcohol soluble material was further purified with ion-exchange columns consisting of 1 mL each of Amberlite IRA-67 (acetate form) (Sigma, St. Louis, MO, USA) and Dowex 50W (hydrogen form) (Sigma). The filtrate then contained neutral carbohydrates and was free of charged materials. The extract was evaporated to dryness at 55 °C under vacuum (Rapidvap Vacuum Evaporation System; Labconco, Kansas City, MO, USA) and redissolved in 10 mL of high-performance liquid chromatography (HPLC)-grade water.

2.4. Determination of Soluble Sugars

After filtration (0.22 µm) and appropriate dilution, samples were subjected to high-performance anion exchange chromatography (Dionex, Sunnyvale, CA, USA) with pulsed amperometric detection (ED50; Dionex) in a device equipped with a Carbopac PA-1 column (CarboPacTM PA1 Analytical, 4 × 250 mm; Dionex) to resolve and quantify soluble sugars. Carbohydrates were eluted with 100 mM NaOH at a flow rate of 1.0 mL min⁻¹ for 20 min [28]. The separated carbohydrates (sucrose, glucose, fructose, mannose and myo-inositol) were quantified by comparison with known standards. System control, data acquisition and processing were performed with PeakNet (5.1) software (Dionex, Sunnyvale, CA, USA), as described by Ranwala and Miller (2008) [27].

2.5. Determination of Starch

The residue from the ethanol extraction was reserved for starch analysis as described by Hou [28]. The residue was boiled for 6 h to gelatinize the starch. After cooling, 1 mL of Na-acetate buffer (pH 4.5, containing 50 units of amyloglucosidase) was added to each dried sample, which was then incubated for 24 h at 55 °C. After incubation, the samples were filtered and diluted to 10 mL with HPLC-grade water. The subsequent procedure was similar to that of the soluble sugar analysis described previously. The amount of glucose released was determined by high-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) using an aliquot of the digested sample. The amount of starch was estimated according to the amount of glucose released.

2.6. PCA

Principal component analysis (PCA) was performed by the statistics function prcomp within R (www.r-project.org (accessed on 15 March 2023)). The data were unit variance scaled before PCA.

2.7. Statistical Analysis

Statistical analyses of the morphological and physiological parameters were performed using one-way analysis of variance (ANOVA) and Duncan’s multiple range test (DMRT) to compare the differences among different samples and treatments. Differences between mean values were subjected to the least significant difference test using a statistically significant level set at p < 0.05. Correlation analysis using the scale morphological and physiological parameters and bulblet formation parameters of the three cultivars was also conducted using Pearson’s two-tailed tests. All computations were performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA). Illustrations were generated using GraphPad Prism (GraphPad Software, Inc., 8.0, San Diego, CA, USA) and PowerPoint software (Microsoft Office 365 ProPlus, Redmond, WA, USA).

3. Results

3.1. Morphological Analysis of Scales in Three Lily Cultivars

By analyzing the length, width and height of the scales of the three lily cultivars, we found that the scale width of the three cultivars showed a decreasing trend from the outer
scale (OS) to the inner scale (IS), and the scale width was distributed in the range of 15 mm to 35 mm (Figure 1a). Notably, the average scale length of NA was significantly lower than that of SOR and YE; however, no obvious pattern was observed from OS to IS in terms of scale length in all three cultivars (Figure 1b). Although the average scale length of NA was the smallest among the three cultivars, the scale thickness of NA was the greatest among the cultivars. In contrast, YE, which had the largest average scale length, showed the smallest average scale thickness (Figure 1c).

Lily bulbs grow by increasing the number and weight of scales. In the preliminary experiment, we found that the ability to regenerate bulblets was rather weak for the newly formed ISs. Thus, only the OS and middle scale (MS) were analyzed in the current research (Figure 1d). To test whether OS and MS can be distinguished by morphological characteristics alone, principal component analysis (PCA) was performed (Figure 1g). The results showed that the MS and OS of the three cultivars were significantly separated on the principal component 2 (PC2) axis with a contribution rate of 24.47%, while NA was significantly separated from SOR and YE on PC1 (49.5%) (Figure 1g). However, when the scale mass values (such as fresh weight (FW) and dry weight (DW) of scales) were included in the PCA, MS and OS could not be separated on PC1 or PC2 (Supplementary Figure S2). Since MS and OS can be distinguished according to their morphological indexes (Figure 1g), we took MS and OS as the objects for subsequent comparative analysis among the three cultivars.

3.2. Analysis of Morphological Differences during Scale Propagation

To determine how light conditions affect the occurrence of bulblets, we conducted continuous observation of scale propagation under different light conditions (Supplementary Figure S1), mainly considering two aspects: the morphological changes in the scales and the state of regenerated bulblets.

3.2.1. Morphological Changes during Scale Propagation under Dark Conditions

From day 4 of culture, the scales of all the three cultivars were oxidized and turned brown at the separation of the basal plate. The NA scales were the first to undergo morphological changes, and the morphological changes of OS in the three cultivars were all earlier than those of MS. The base of NA_OS gradually expanded from day 7 and meristematic domes were formed on the base of scales from day 9. The meristem continued to expand from day 11 to 13, during which root formation (2 to 4 roots per scale) was observed at the scale bottom. Newly differentiated bulblets with a clearly recognizable scale structure were observed by day 19 and continued to expand from day 19 onwards. Unlike NA, the scales of SOR and YE did not undergo significant morphological changes before day 11 but likewise developed differentiated bulblet structures by day 19. Notably, the color change of scales was not obvious under dark conditions, and milky white (NA) and purplish red (SOR and YE) coloration was maintained during the scaling process. The newly formed bulblets of the three cultivars were all milky white and had 1–2 finer roots with only a few root hair structures by day 60 (Figure 2a).

3.2.2. Morphological Changes during Scale Propagation under Light Conditions

In contrast to dark conditions, under which the scales remained the same color, scales turned purplish red on the fourth day under light conditions. From day 7 onwards, the base of the scales gradually turned green, and this trend was more pronounced in OS than in MS. NA was still the first to start to differentiate and meristem initiation started to be active at the base of scales at day 9; however, meristems initiated earlier in SOR and YE under light conditions than under dark conditions. From the 13th day to the 19th day, as the color of the scales gradually deepened, bulblet structures formed and continued to expand. Notably, no significant differences were observed in the number of roots formed at the base of scales under light conditions compared to that under dark conditions. However, the roots formed under light conditions were longer and thicker and had more root hairs than those formed under dark conditions (Figure 2a). The newly formed bulblets were
green with some purplish red scales (Figure 2a). In addition, the newly formed bulblets of SOR scales sprouted multiple leaves under light conditions (Figure 2a). In conclusion, there were significant morphological differences in scale propagation under light and dark conditions, which were mainly reflected in scale color changes, root morphology and the status of newly formed bulblets.

Figure 2. Bulblet regeneration under dark and light conditions. (a) Morphological comparisons of outer scales from three lily cultivars between dark and light conditions at day 60. Bar = 1 cm. Bulblet regeneration rate of scales from three lily cultivars under dark (b) and light conditions (c). (d) Number of regenerated bulblets under dark and light conditions. Fresh weight (e) and dry weight (f) of a single regenerated bulblet from light and dark conditions. Data are represented as the means ± standard error of mean (SEM) (n = 6 biological replicates). FW, fresh weight; DW, dry weight; ns, no significant differences at p < 0.05. Different letters indicate significant differences at p < 0.05 according to DMRT. Asterisks indicate significant differences between light and dark conditions (DMRT, ** p < 0.01).

3.3. Comparative Analysis of Incidence Rate and Regeneration Efficiency of Scale Propagation

The change trend in the bulblet regeneration rate was significantly different between light and dark conditions (Figure 2b,c). Under dark conditions, the bulblet formation stage of the three cultivars was distributed from days 9 to 13, while the formation stage of the bulblets was more concentrated (days 9–11) under light. The bulblet regeneration rate was substantially accelerated under light conditions compared with dark conditions. For example, NA_OS had a 100% regeneration rate by day 13 under light (Figure 2c), but the regeneration rate of NA_OS did not reach 100% under dark until day 33 (Figure 2b). Moreover, the regeneration rate of NA_MS reached 100% on day 19 under light conditions (Figure 2c), while within the same period under dark conditions, the regeneration rate of NA_MS was only 63.5% (Figure 2b).

Figure 2d shows that light conditions had a significant effect on the number of regenerated bulblets, and the number of regenerated bulblets obtained under light conditions was significantly higher than that under dark conditions. Moreover, the number of regenerated bulblets in OS was significantly higher than that in MS under light conditions (Figure 2d). The number of bulblets regeneratated by OS was also significantly higher than that regenerated by MS under dark conditions, except for SOR (Figure 2d). Light had a certain effect on the bulblet regeneration ability in MS. Notably, light significantly increased the number of regenerated bulblets in NA_MS (Figure 2d). Moreover, no significant differences were found in the individual FW and DW of bulblets between dark and light conditions.
(Figure 2e). However, the weight of regenerated bulblets derived from OS was significantly higher than that from MS in the dark, except for YE.

These findings indicated that light conditions had no significant effects on the number of regenerated bulblets but significantly influenced the initial formation time and formation speed of bulblets. The formation period of bulblets was more concentrated and the formation speed was faster under light conditions than under dark conditions. In addition, light significantly increased the number of regenerated bulblets but had no obvious effect on the weight of individual bulblets of OS.

3.4. Effects of Explant Morphological Characteristics on Bulblet Regeneration

3.4.1. Comparative Analysis of Morphological Indexes between OS and MS in Three Lily Cultivars

PCA can distinguish OS from MS (Figure 1g). Specifically, there was a significant difference in scale width between OS and MS, and the width of OS was significantly larger than that of MS in all three cultivars (Figure 3a). SOR had the largest scale width compared to the other cultivars for both its OS and MS (Figure 3a). Notably, although the scale length of NA_OS was significantly greater than that of NA_MS when compared within cultivars, there was no significant difference in scale length between OS and MS when comparing the three cultivars overall (Figure 3b). There was also a significant difference in scale thickness between OS and MS when considering the three cultivars overall (Figure 3c). Although YE_OS showed the largest average scale thickness, there was no significant difference between YE_OS and YE_MS. Interestingly, morphological indexes related to scale width, including basal area (BA) (Figure 3d), facial area (FA) (Figure 3e) and volume (Figure 3f), showed significant differences between OS and MS in all three cultivars. Notably, SOR_OS showed a prominent size advantage over the other cultivars. In addition, significant differences were also observed between OS and MS in the length-to-width ratio (LWR) and width-to-thickness ratio (WTR) of scales (Figure 3g,h).

![Figure 3. Statistical analysis of the morphological characteristics of scales. (a–h) Statistical analysis of the morphological characteristics of outer and middle scales from three lily cultivars before scaling. (a) Scale width. (b) Scale length. (c) Scale thickness. (d) Scale basal area. (e) Scale facial area. (f) Scale volume. (g) Length-to-width ratio of scales. (h) Width-to-thickness ratio of scales. Different letters indicate significant differences at p < 0.05 according to DMRT. Asterisks indicate significant differences between OS and MS (DMRT, ** p < 0.01, * p < 0.05). ns, no significant differences at p < 0.05. Scale basal area = width × thickness, Scale facial area = width × length, Scale volume = width × length × thickness. OS, outer scale; MS, middle scale.](image-url)
3.4.2. Correlation Analysis of Scale Morphological Indexes with the Quantity and Quality of Regenerated Bulblets

Since light condition affects the number of regenerated bulblets (Figure 2d), we then conducted correlation analysis using the data under dark culture conditions to explore the influence of scale morphological indexes on the quantity and quality of regenerated bulblets (Figure 4b). OS and MS differ in their morphological characteristics (Figure 1g). Therefore, the first and critical question was to assess whether the different scale sizes could account for the observed bulblet regeneration differences. This was addressed by analyzing the length, width, and thickness of a single scale, thereby converting the morphological characteristics to numerical traits. The number of formed bulblets (BulbletNO) and FW and DW of bulblets were used as the basic parameters for conducting correlation analyses with the scale morphological indexes.

Figure 4. Correlation analyses between scale morphological characteristics and the status of regenerated bulblets. (a) Morphological comparison of regenerated bulblets from outer scale (OS) and middle scale (MS) under dark conditions. Each group contains regenerated bulblets derived from five scales. (b) Correlation analyses between scale morphological characteristics and status of regenerated bulblets. * represents significant at $p < 0.05$, ** represents significant at $p < 0.01$. Tissue, indicates scales from outer and middle layer of bulb. FA, scale facial area; BA, scale basal area; LWR, Length-to-width ratio of scales; WTR, width-to-thickness ratio of scales; BulbletFW, bulblet fresh weight; BulbletDW, bulblet dry weight; BulbletNO, number of regenerated bulblets. Words in red indicate positive correlations and words in green indicate negative correlations.

The results revealed that BulbletNO (0.809 **), FW of bulblets (0.661 **) and DW of bulblets (0.441 **) were all significantly positively correlated with tissue (Figure 4b). Moreover, the older the physiological age of the scales was, the more bulblets could form, and the heavier the mass. In addition, scale width (0.579 **) and SA (0.429 **) were significantly positively correlated with scale physiological age, while the LWR of scales ($-0.610 **$) was significantly negatively correlated with scale physiological age, and scale length was not significantly correlated with scale physiological age. In addition, the number of formed bulblets was significantly positively correlated with scale width (0.581 **), FA (0.544 **) and WTR (0.437 **), significantly correlated with scale length (0.366 *), and significantly negatively correlated with LWR ($-0.379 *$). Additionally, the weight of the formed bulblets was significantly positively correlated with scale width (0.627 **), volume (0.540 **) and FA (0.593 **), and was significantly correlated with scale length (0.392 *) and BA (0.407 *). In contrast, the FW of bulblets was significantly negatively correlated with LWR ($-0.390 *$).

It indicated that the longer the scale length is and the shorter the scale width is, the more unfavorable the conditions are for the occurrence and nutrient accumulation of bulblets. Scale width may play a key role in the occurrence of bulblets and their nutrient...
accumulation. The larger the scale FA is, the more favorable the conditions are for the occurrence of bulblets and their nutrient accumulation. In addition, BA had no significant correlation with the number of newly formed bulblets.

3.5. Effects of Explant Nutritional Status on Bulblet Regeneration of Three Lily Cultivars

3.5.1. Analysis of Initial Nutrient Composition in Different Scales

Among the cultivars, starch and soluble sugars accounted for 50–80% of scale DW. The concentration of starch varied from 313.07 mg g\(^{-1}\) DW to 600.42 mg g\(^{-1}\) DW (Figure 5a). Generally, MS contained significantly higher levels of starch than OS (except for SOR, Figure 5a). No significant differences were found among MS samples in the three lily cultivars (Figure 5a). By measuring the initial nonstructural carbohydrate content in scales, we also found that the concentration of sucrose (Figure 5b) was always greater than that of glucose (Figure 5c), fructose (Figure 5d), mannose (Figure 5e) and myo-inositol (Figure 5f) in all the assessed lily scales. Notably, OS contained significantly higher levels of sucrose than MS (Figure 5b). The highest average content of sucrose was found in the OS of NA (161.71 mg g\(^{-1}\) DW), followed by the OS of YE (127.61 mg g\(^{-1}\) DW). The contents of fructose (Figure 5c), glucose (Figure 5d), mannose (Figure 5e) and myo-inositol (Figure 5f) in the OS of YE were all significantly higher than those of the other scales. The sucrose:starch ratio (RSS) of NA_OS and YE_OS was significantly higher than that of SOR, while the RSS of SOR had no significant difference between OS and MS. The hexose-to-sucrose ratio (RHS) of YE_OS was the highest and significantly higher than that of YE_MS and the other two cultivars, while there was no significant difference between OS and MS for NA and SOR.

![Figure 5](image)

**Figure 5.** Statistical analysis of the initial nutrient status of scales from three lily cultivars before scaling. (a) Starch. (b) Sucrose. (c) Fructose. (d) Glucose. (e) Mannose. (f) Myo-Inositol. (g) Sucrose-to-starch ratio. (h) Hexose-to-sucrose ratio. Different letters indicate significant differences at \(p < 0.05\) according to DMRT. OS, outer scale; MS, middle scale.

3.5.2. Correlation Analysis of Scale Nutritional Status with the Quantity and Quality of Regenerated Bulblets

To determine whether the initial content and change rate of nutrients in scales affect the occurrence of bulblets, correlation analysis was conducted (Figure 6). The results showed that the contents of sucrose (0.596 **), fructose (0.419 *), glucose (0.462 **) and mannose (0.510 **) were all significantly positively correlated with the physiological age of scales, whereas starch (−0.413 *) was significantly negatively correlated with age. In contrast, the starch and sucrose contents had no significant correlations with cultivar, and there was no significant correlation between cultivar and the number or weight of bulblets.
The number of bulblets was significantly positively correlated with the content of sucrose (0.411 **), fructose (0.473 **), glucose (0.477 **), mannose (0.457 **) and myo-inositol (0.441 **), while positive correlations were also observed in RHS (0.442 **) and RSS (0.510 **). Notably, RSS was significantly positively correlated with the FW (0.486 **) and DW (0.480 **) of bulblets (Figure 6). Moreover, changing ratio of the content of fructose (RFru) and changing ratio of the content of glucose (RGlc) were significantly positively correlated with both the FW and DW of bulblets (Figure 6). In conclusion, the sucrose content, the sucrose-to-starch ratio and RGlc were all significantly correlated with the quantity and quality of regenerated bulblets, which is worthy of further investigation.

### Figure 6. Correlation analyses between nonstructural carbohydrate contents in scales and the status of regenerated bulblets

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<th>Bulblets</th>
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### 4. Discussion

#### 4.1. Morphological Characteristics of Scales from Different Positions of the Bulb May Potentially Influence Bulblet Formation during Scaling

Commercially grown ornamental geophytes are propagated by vegetative means to maintain genetic purity [4, 5, 22]. For conventional commercial vegetative propagation of lily, bulblets are produced from scales [12, 29]. An efficient scaling technique consists of detaching the bulb scales and culturing them in moist and warm conditions for several weeks, during which internal and external factors cooperate to promote the formation of bulblets. Contemporary research on scaling has largely focused on external treatments (both physiological and chemical) for technique improvement. In contrast, much less is known about the influence of the morphological traits of the explant on the regeneration capacity during scale propagation. In the present study, we analyzed the scale propagation process in three lily cultivars, focusing on the correlation between morphological characteristics of scales and bulblet regeneration capacity. PCA was used to linearly transform multiple morphology-related variables into a few comprehensive variables through dimensionality reduction of the data. Through PCA, we successfully separated the scales from different positions of the bulb into two groups (OS and MS) (Figure 1g), in which the morphology-regeneration relationship could be precisely investigated.

Genotype is an important factor affecting scale propagation in lily [30]. Scales from various genotype backgrounds differ in thickness and nutrient contents, thus influencing
the number and biomass accumulation of differentiated bulblets. However, we found that the tissue source and scale width of a single scale had more significant effects on reproduction than differences in cultivar. In our study, no significant correlation was found between the number and quality of regenerated bulblets and the bulb cultivar (Figure 4b). However, tissue source was significantly correlated with the status of regenerated bulblets (Figure 4b), and as the physiological age of scales increased, the number and weight of regenerated bulblets both significantly increased. This finding is contrast to in vitro propagation of bulblet, during which younger explant often had higher bulblet productivity than old ones [31].

In this study, we found that bulblet regeneration ability increased significantly with scale width. However, no significant correlation was observed between the number of regenerated bulblets and scale BA (Figure 4b), which is different from what was previously reported by Sun [32]. Intriguingly, we found that both the quantity and quality of bulblets were significantly positively correlated with scale FA (Figure 4b). Previous research reported that the ability to form bulblets was closely related to the position of the single scale on the bulb [9,14,29,33,34]. It is generally believed that OS and MS have stronger reproductive ability [13,32], because IS has relatively small BA and weak accumulation of nutrients, which cannot provide sufficient energy for the differentiation of bulblets during scaling. The vascular bundles in lily scales are distributed parallel to the scale growth trend; thus, the wider the base of the scales is, the more vascular bundles there are, and the greater the potential to produce regenerated bulblets. However, it remains to be further explored why the number of regenerated bulblets is significantly positively correlated with FA, e.g., whether the length of the vascular bundle in scales also affects the ability to produce bulblets.

To date, much attention has been given to the overall size of bulbs. Commonly, a bulb’s size is determined by measuring its circumference. A circumference of at least 12 cm denotes ‘large’ bulbs, while bulbs less than 10 cm are considered ‘small’ [35]. However, the effect of the size of a single scale on the reproduction coefficient and weight of regenerated bulblets has not been fully investigated. Under in vitro conditions, the size of the explant has a major effect on the size of the regenerated bulb [35], and the size of the bulblets produced in vitro has a strong effect on performance after planting [36]. Moreover, basal plate is a necessary part for explant to regenerate bulblet in the Amaryllidaceae family [4,37,38]. The more layers of scales connecting to a basal plate (e.g., two-scale, tri-scale and four-scale), the better performance in bulblet multiplication of explant [39–41]. In this respect, the efficiency of bulbing could be affected by morphological traits of explant. The single scale propagation system of lily is a suitable model to study the internal factors influencing bulblet formation. Therefore, it is very important to study the morphological characteristics of the single scale explant and its relationship with the formation of bulblets in lily during scale propagation. In this study, comparative studies between scales from different positions of the bulb allowed us to identify representative morphological traits of scales with potentially high regeneration capacity (Figures 3 and 4). Explants with relatively larger width and lower LWR have a better competence for bulblet regeneration, which is highly informative for graded scale propagation of lily and hence improvements in bulb yield.

4.2. The Initial Content of Nonstructural Carbohydrates Correlates with Variations in Regeneration Capacity during Scaling

Lily scales have the capacity to regenerate new meristems that develop into bulblets (without the supply of exogenous nutrients) when they are detached from the mother bulb and incubated in suitable conditions [5]. Moreover, unlike bulbous flowers such as Lycoris [42], lily can propagate vegetatively via a single scale without relying on the basal plate structure. This unique capacity makes lily scales a potential good candidate to explore factors inherent to the explant that affect regeneration. The scale propagation of lily is a process in which the starch in mother scales is decomposed into soluble sugar under the action of amylase to supply the formation and development of bulblets. Therefore, the
metabolic characteristics of starch, the major storage polysaccharide in Lilium bulbs [43,44], are closely related to the formation and development of bulblets [11,45], which is consistent with the swelling of storage organs in potato and Tulipa edulis [46–49]. Here, we found that the average starch content of OS was significantly lower than that of MS, whereas the sucrose content in OS was significantly higher than that in MS (Figure 5a). Based on these results, we proposed that efficient starch degradation rather than accumulation in scales before scaling might be beneficial for efficient bulblet regeneration.

In this study, we found that nonstructural carbohydrates accounted for 50–80% DW in all the lily scales investigated. Although the starch and sucrose contents varied among different cultivars, they differed significantly among different tissues (Figure 5a,b). This is consistent with the differences in physiological functions (being consumed and about to be consumed) of scales located at different positions of the bulb. Generally, starch catabolism in OS is vigorous, and nutrients are consumed preferentially to supply the growth and development of the bulb. Previous studies have reported that carbohydrate status might be one of the regulatory signals that allows plants to perceive and respond to changes in the environment and to different developmental stages [50,51]. Ren [23] proposed that sugar dynamics might be good indicators of the metabolic processes that lead to phase transition towards bulblet regeneration of Lycoris. A high ratio of hexose to sucrose likely supports cell division in the bulblet formation stage [23]. In the present study, it should be noted that the initial RSS was significantly positively correlated with the quantity and quality of bulblets (Figure 6), suggesting that the RSS might indicate the reproductive potential of scales. In addition, correlation analysis showed that the weight of the bulblet was positively correlated with the change ratio of Fru and Glc, which was consistent with the increase in reducing sugar content during bulblet regeneration. We also found that the number of regenerated bulblets was significantly positively correlated with the initial contents of fructose, glucose, mannose and myo-inositol; (Figure 6), thus, it is necessary to further explore the effects of these soluble sugar components on the formation of bulblets during scaling.

4.3. Light Accelerated the Formation of Bulblets during Early Scale Propagation

Light treatment influences the morphogenesis of bulblets, but the results of previous studies vary [29]. To date, much attention has been given to the effects of light treatment on bulblet regeneration under in vitro conditions [34,52–54], whereas research on light during scale propagation is still limited. In this study, we found that light had no effect on the incidence of bulblets, but the formation of bulblets was advanced and better morphological consistency of bulblets could be obtained under light conditions (Figure 2b,c). Cao [24] noted that light had a significant effect on the number of differentiated bulblets, as well as on the quality of bulblets. Although we also found that the number of regenerated bulblets under light conditions was significantly higher than that under dark conditions, no significant effect of light treatment on the FW/DW of a single bulblet was observed, which might have resulted from the limited observation period (60 days).

Notably, light contributed to a significant increase in the weight of bulblets derived from MS during the short incubation period (Figure 2e,f). We also found that under light conditions, stronger roots and leaves were formed from the differentiated bulblets, the scales of which turned green, contributing to nutrient accumulation via photosynthesis (Figure 2a). However, the effect of bulblets formed under different light conditions on the later growth and development of the bulb and the quality of cut flowers remains to be further explored. On the basis of the above results, we suggested that proper light treatment during the formation stage could accelerate and promote the formation of bulblets, improving the uniformity of regenerated bulblets and obtaining more bulblets. We hope to provide a reference for improving reproduction efficiency by taking advantage of proper light treatment for bulb production by scaling. Elucidating the details will still be a challenging task for follow-up studies. However, incremental research progress promotes slightly more productivity in bulbs.
5. Conclusions

Scaling, during which bulblets form without an exogenous supply of nutrients and plant growth regulators, is a useful model for investigating factors contributed to regeneration discrepancies inherent to the explant. In this study, morphological characteristics, nutritional status and light treatments that potentially influence bulblet formation were identified. Scales from different positions of donor bulb could be clearly separated by morphological characteristics via PCA. Explants with relatively larger width and lower LWR have a better competence for bulblet regeneration. A higher initial ratio of sucrose to starch in scales was more conducive to the bulblets formation. Formation of bulblets was positively enhanced by light treatment and better morphological consistency of bulblets was obtained. Our work provides comprehensive morphological and nutritional analyses of scaling propagation which will pave the way for future research into bulblet formation at the molecular level and will provide useful information for graded scaling-based breeding and bulb production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13071341/s1, Figure S1: Morphological observations under dark and light conditions during scaling propagation. Figure S2: Principal component analysis (PCA) of morphological and weight characteristics of scales from different position of three lily cultivars.

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