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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Factors affecting bulblet growth of *Lilium sp*. via *in vitro* － modulating the links between organogenesis and methodology
(*In vitro* におけるユリ属植物の子球の成長に影響する要因 － 器官形成制御の方法)

学位論文要約：
Dissertation Summary

Lily — the geophyte is an excellent model for studying the fundamental relationship for bulb production *in vitro*. The *in vitro* propagation technique of lily bulb production through scale explant culture is the prolific vegetative propagation method as an alternative to conventional methods for producing lilies. Because of their commercial value, maintaining their genetic purity is required for commercially grown cultivars, so they are usually propagated by vegetative means. As a monocot and bulb-scale plant, the natural vegetative propagules are small bulblets, either produced above ground on the stems (bulbils) or underground on bulb scales (Kumar et al., 2006) or on artificial medium via *in vitro* pathway. In general bulb production under conventional process is done by 'scaling' (scaling—excised scales are placed in moistened vermiculite to produce small bulblets generally, 1–4 per scale) (De Klerk et al. 1992; Langens-Gerrits and De Klerk, 1999). In the course of scaling, the process is relatively very slow and requires a longer period of time for the flower bulb production, ontogenic development with bulblet size and introduction of newly bred cultivars (Langens-Gerrits and De Klerk, 1999).

In contrast, similar to scaling *in vitro* propagation technique of lily bulb production through scale explant on artificial culture medium supplemented with all essential nutrients and carbon source is the prolific vegetative propagation method (Bahr and Compton, 2004) used by commercial growers to produce large numbers of high-quality and disease-free plantlets in a short period of time (George et al., 2008). Since the late 1970s, *in vitro* culture techniques have been applied for the multiplication of propagules of lilies because of high regeneration potential (Takayama and Misawa, 1979, 1983; Chang et al., 2000; Skorić et al., 2012). Advancement in the field of *in vitro* culture techniques of lilies evolved

to provide an alternative to pervious conventional methods for bulblet multiplication because of the advantages of i) high regeneration potential (the potency) of lily bulb scale (swollen petiole) tissues (Van Aartrijk and Van der Linde, 1986; Bahr and Compton, 2004; Han et al., 2005), ii) several folds increased multiplication rate of high-quality plantlets in a short period of time (George et al., 2008), and iii) clean planting material (Skorić et al., 2012). Moreover, the small scales excised from the new bulblets produced *in vitro* can be used further as a new starting material so that per year a few propagation cycles can be performed. However, the drawbacks of micropropagation are similar to the conventional process and that is the small size of the produced propagules (bulblets) (Kumar et al., 2001) leads to suboptimal performance after planting in soil.

Recent studies reported that bulb size is an important factor for floral transition (Lazare and Zaccai, 2016), and *in vitro* factors ripples lily bulblet size to enhance *in vivo* performance (Islam et al., 2017). Hence, clear understanding the growth mechanism of lily bulblet *in vitro* is the key feature, which is the most important factor towards understanding 'how lily bulblets achieve growth *in vitro*?' would be helpful to improve the optimal growth conditions of lily bulblets within the *in vitro* pathway. Regardless of advantages, the major problem with *in vitro* propagation is small bulblet size and this initial size of bulblets not only strongly affects growth and morphogenesis rates but also the transition between various vegetative and reproductive phases of lilies during development after planting and so, makes it a highly complex process. For that reason, various studies were done on techniques, to analyze the complex multivariate data sets to break down the complexity in a simpler means to a better understanding of the complex effects of the variables involved during the *in vitro* bulb production on the *in vitro* culture and the *in vivo* performance to an improvement of the pathway. Hence, the aim of this study was; i) to identify the factors affecting bulblet growth of *Lilium sp*. via *in vitro*, ii) modulating the links between organogenesis and methodology, and iii) production of high quality (large and uniform) bulblets *in vitro*.

Factors affecting bulblet growth of *Lilium sp.* — *in vitro* and *in vivo*

In **Chapter 2**, to understand how lily bulblets regenerating from scale explants do grow *in vitro*, a detailed study on the effect of different sucrose concentrations on bulb formation and the growth *in vitro* and their performance in soil over the growing season was done to reveal the effective *in vitro* culture condition to enhance *in vivo* performance of lily bulblets. The effective *in vitro* factors

to enhance *in vivo* performance of lily bulblets the results showed that bulblet growth correlates with the carbon reserves inside explant tissue and in the medium. The internal storage of starch plays a vital role and influences regeneration and bulblet growth in both *in vitro* and *in vivo*. There is a high correlation (R^2= 0.9672) between bulb size (weight) after the growth season at planting and initial bulb size (weight gained *in vitro*). A clear understanding of the factors affecting bulblet growth of lilies *in vitro* and *in vivo* would facilitate to improve the optimal growth process in the pathway (Fig. 1).

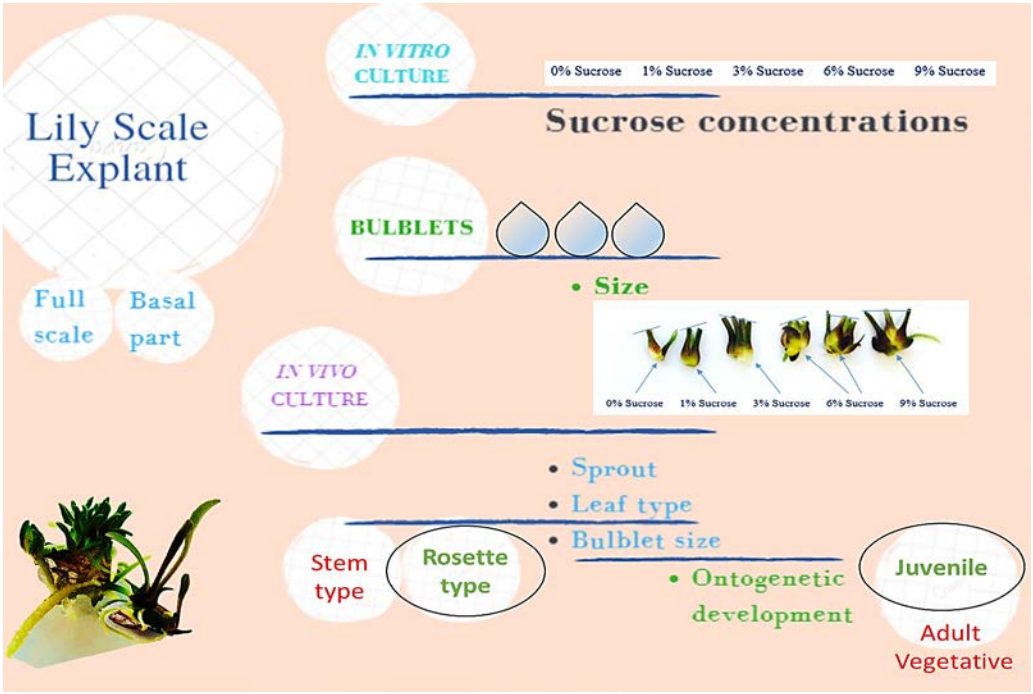

Figure 1. Factors affecting bulblet growth of lilies *in vitro* and *in vivo*.

In conclusion, the main factors determining the growth of bulblets *in vitro* are explant size and sucrose concentration in the medium. Furthermore, internal storage and transport of sucrose plays a vital role and influences regeneration and bulblet growth. The size of the bulblets produced *in vitro* strongly affects performance after planting. From a horticultural point of view, we recommend culturing lily scale explants in a high sucrose concentration during regeneration of bulblets *in vitro* to increase bulblet size, which is the main point of interest for future production.

Factors affecting bulblet growth of *Lilium sp.* – a correlative assessment of culture media *in vitro*

In **Chapter 3**, we investigate a correlative assessment of different culture media *in vitro* to determine the effect of culture media on enhancing *Lilium* bulblet size, the key to successful culturing of lilies *in vitro* and gain more information about bulblet production *in vitro*. The correlative

assessment of culture media showed that explant regeneration percentage was higher for the explants on the solid medium and concomitantly, took fewer days to regenerate. Therefore, the results of bulblet performance (bulblet number and size by fresh weight) including the root:shoot ratio (Fig. 2) indicate that the liquid static culture with medium renewal ensures the avoidance of nutrient depletion, in particular, sucrose, and therefore might be a useful technique for producing bulblets and enhancing bulblet growth *in vitro* compared with those explants grown on an agar solidified medium. Accordingly, we conclude that *in vitro* bulblet regeneration and growth of *Lilium* spp. is not only influenced by sucrose concentration in the basal medium but also by various forms of culture media. However, this performance of bulblets in liquid static culture was influenced by the renewal of medium and thus, avoiding nutrient depletion compared with bulblets grown on an agar solidified medium *in vitro*.

Solid medium (T_1) Semi-solid medium (T_2) Liquid medium (T_3)

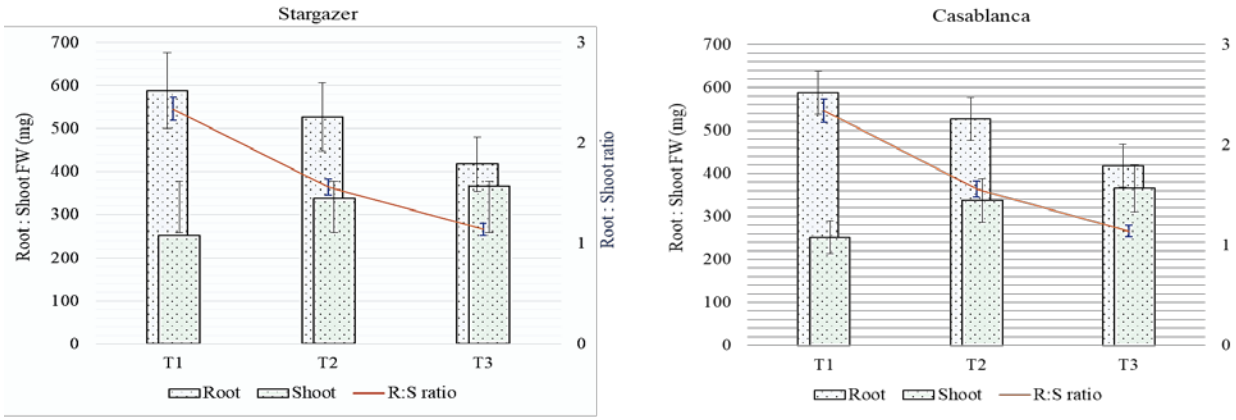

Figure 2. Influence of culture medium on the root:shoot ratio of lily *in vitro*.

(Explants were cultured *in vitro* for 90 days under standard conditions and the root:shoot ratio (fresh weight) was determined. Each data point represents the average ± SE. T_1, T_2, and T_3 represents solid, semi-solid, and liquid medium, respectively.)

Factors affecting bulblet growth of *Lilium sp*. — an upscale modeling of explant placement *in vitro*

In **Chapter 4**, in the pathway, a novel method focusing on culturing lily bulb scale explant placement on culture medium to determine the influences on regeneration and organogenesis of lilies to understand related bulblet growth mechanism and to establish an upscale model of bulb scale explant placement on culture medium *in vitro*. Combining the results with morphological upscaling of explants to regeneration and organogenesis of lily influenced by explant placement on the medium *in vitro* visually indicates that vascular tissue deep explant placement (T_2) influences uptake of the required sugar from the medium to reinforce and therefore, faster regeneration and rapid multiplication (organogenesis/bulblet production). On the other hand, lily bulb scale explants subjected to adaxial surface deep (T_1) placement on culture medium *in vitro* had slower progress to regeneration and organogenesis due to the small amount of tissues exposed to the medium and therefore, lower uptake of the required sugar from the medium. Fully submerged explant placement (T_3) failed because of the anaerobic condition. Moreover, these measurable features may still be amenable for detecting more subtle explant source differences that will support a more direct testing of growth difference effects detected by placement on plant growth *in vitro* (Fig. 3). Accordingly, we conclude that explant placement on the medium, chlorophyll content in the explant tissues, and CO_2 within the glass vessel affect lily bulb scale explant regeneration and organogenesis *in vitro*.

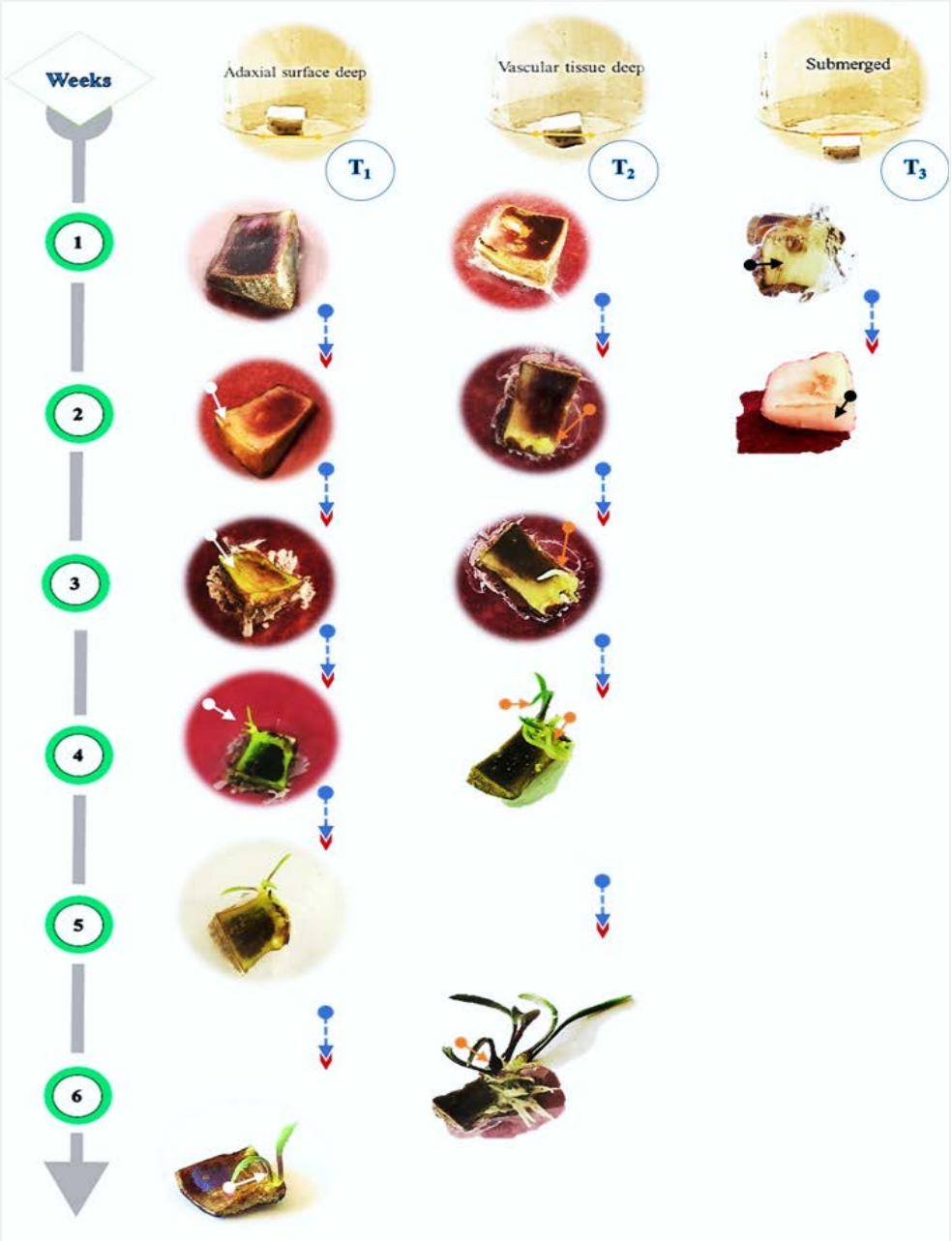

Figure 3. Morphological progression of explants to bulb production of lily influenced by explant placement on medium *in vitro*

(Explants placement: T_1, adaxial surface deep; T_2, vascular tissue deep; T_3, fully submerged)

Factors affecting bulblet growth of *Lilium sp.* — tracking ontogenic development and bulb production *in vitro*

In **Chapter 5**, a detailed study on the poor growth of bulblet and ontogenic development and which are the major problems of adventitious bulb production of lilies *in vitro* through scale explant culture. Hence, the study describes an effective *in vitro* culture process for lily to track bulblet growth, ontogenic development, and bulb production. In the results, there is a threshold weight (bulblet size) for

ontogenic development was about 300 mg at which 100% ontogenic development occurred (Fig. 4) and confirms that avoiding depletion of sucrose in the media is the key to achieving desired bulblet growth and ontogenic development *in vitro*. Bulblet performance was affected by the amount of medium because of the increasing amount of sucrose *in vitro*, and a low temperature during the culture period acts as a signal for inducing ontogenic development. However, the amount of medium for bulblet production *in vitro* is often inadequate because of nutrient depletion, particularly sucrose. Hence, continuous bulblet growth and ontogenic development were affected by sucrose depletion in the media during lily bulblet regeneration *in vitro*. In accordance with bulblet regeneration and growth, sucrose depletion was linear over time as well as being concentration dependent (Fig. 5). Avoiding depletion of sucrose in the media is the key to achieving the desired bulblet growth and ontogenic development *in vitro*. As well as indicates that the bulblets could be sustained and maintained by elevated amount of medium and re-culture.

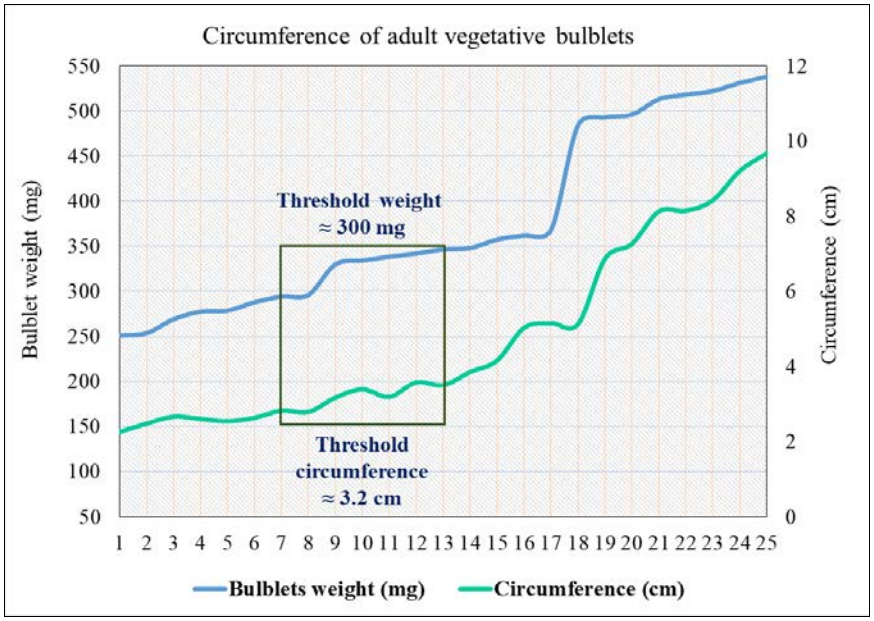

Figure 4. Lily bulblet threshold circumference for phase change

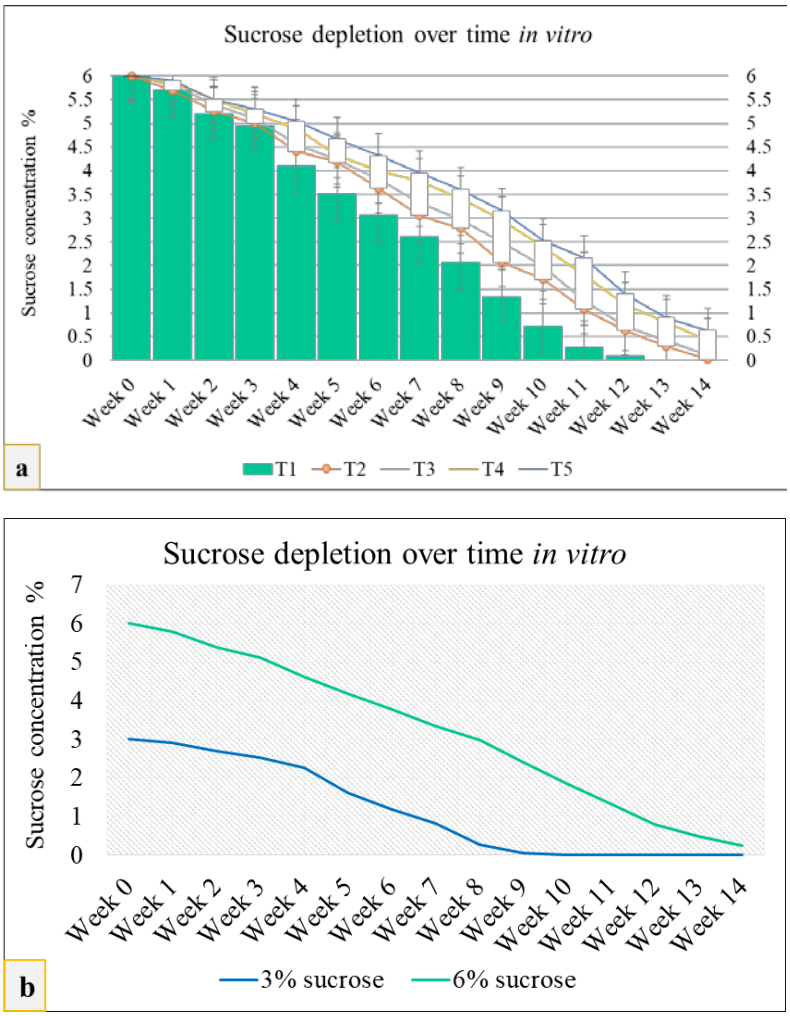

Figure 5. Sucrose depletion over time and amount of medium (a) and sucrose concentration (b) during lily bulblet regeneration *in vitro* (T_1, 10 mL of medium; T_2, 15 mL; T_3, 20 mL; T_4, 30 mL; T_5, 50 mL)

Factors affecting bulblet growth of *Lilium sp.* — a timeline for bulblet to bulb production via *in vitro* and conventional pathways

In **Chapter 6**, the key features from both the *in vitro* and conventional method were taken account to understand and identify pros and cons is associated with bulblet size and time, which is the most important factors. This study describe and compare the performances through morphological characteristics of the lilies, particularly, bulb *in vivo* produced by *in vitro* and conventional culture method, and compare the production timelines *in vitro* vs conventional culture method. Along with the re-culture *in vitro*, the course of bulb growth and ontogenic development was more rapid (1–2 growing seasons to reach the adult flowering phase) because of their bigger initial bulb size and advancement of ontogenic development compare to conventional pathway (3–4 growing seasons). Moreover, this

study represents the first report of lily bulb production through re-culture with a comparison of the timelines and ontogenic development obtained from *in vitro* versus conventional pathway (Fig. 6).

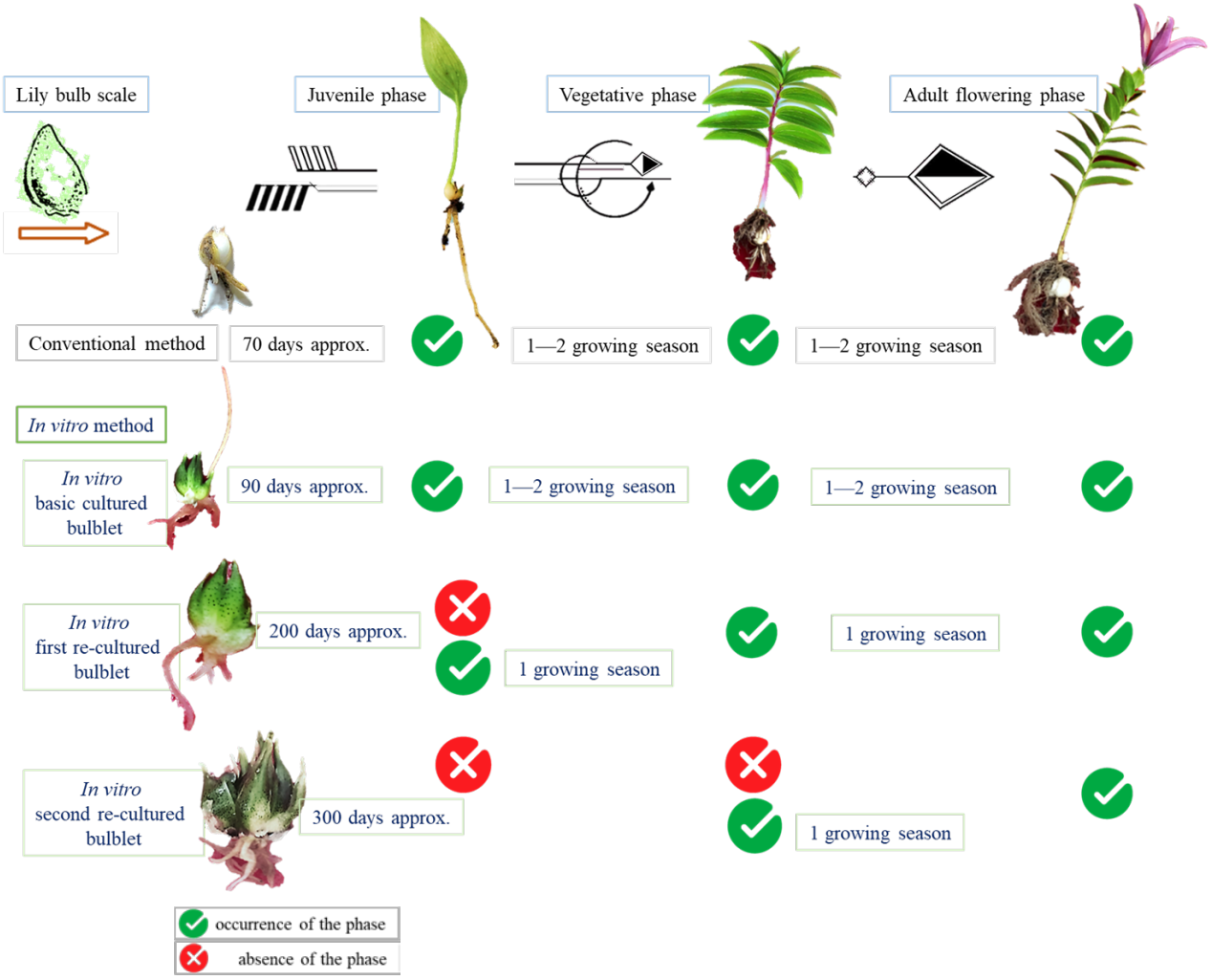

Figure 6. Timeline and course of bulblet growth and ontogenic development through the *in vitro* and conventional pathways in soil

Future Perspectives

The *in vitro* propagation technique of lily bulb production through scale explant culture in the alternative to conventional methods for producing lilies. Hence, there are several *in vitro* protocols for lily bulb production but, most of them are not satisfactorily workable because of inadequate bulb formation, and lack of uniformity in bulb size. And so, research is needed to study and improve *in vitro* bulb formation especially, a clear understanding of the factors affecting *in vitro* regeneration and bulblet growth of lilies and links up through a tidy methodology would be a good use to production of

high-quality bulb. For the reason, this study investigates thoroughly a) to identify the factors affecting bulblet growth of *Lilium* sp. via *in vitro*, b) modulating the links between organogenesis and methodology, and c) for the production of high-quality bulb *in vitro*. It will help to better understand how to produce higher quality bulb rapidly. However, the exact mechanism of sucrose during *in vitro* culture of lily still had to elucidate in detail. A clear understanding about the cold treatment and ontogenic development in the process of lily bulb production also demand interest for future studies. Furthermore, an effect of plant growth regulators in the process of lily bulb production via *in vitro* is still the topic of interest for future research and can be provided the potential to manipulate the pathway.

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