International Journal of Horticultural Science and Technology
Vol. 5, No. 2; December 2018, pp 133-143
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2018.268870.263
Web Page: https //: ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Growth of Lily Bulblets *In Vitro*, a Review

Naser Askari [1,2*], Richard G.F. Visser [2] and Geert-Jan De Klerk [2]
1. Department of Plant Sciences, University of Jiroft, Jiroft, Iran
2. Plant Breeding, Wageningen University & Research, Wageningen,The Netherlands

(Received: 3 November 2018, Accepted: 20 November 2018)

Abstract
In micropropagation of lily, preferably bulblets should be produced: Because bulblets are compact and robust, they are much easier to handle and to plant in soil than shoots. In this review, the various factors that determine bulblet growth *in vitro* are discussed. Gibberellins, jasmonates (JA) and abscisic acid (ABA) are the major identified plant growth regulators (PGRs) for storage organ formation. They also play a major role in lily bulblet growth *in vitro*. Growth conditions such as temperature and light (quantity and quality) strongly affect lily bulblet growth in tissue culture. Moderate abiotic stresses are introduced as new tool to improve storage organ formation *in vitro*. The amounts of endogenous carbohydrates (starch) in the explant and exogenous carbohydrates in the medium (sucrose) influence bulblet growth *in vitro*. It is also discussed how compounds present in the medium or in the scale-explants are translocated to the regenerating bulblet.

Keywords: Lily, *in vitro*, Bulblet growth, Scale explant, Plant growth regulators, Medium components.

Lilium

The genus *Lilium* is one of the *ca.* 220 genera belonging to the *Liliaceae* and comprises about 85 species including many ornamental species. Lilies are among the top 10 commercial flowers of the world. They have large flowers with attractive colors (Fig. 1) and an excellent vase life (Beattie and White, 1993). *Lilium* is characterized by an annual thermo-periodism and is widely distributed in the Northern Hemisphere. *Lilium* is classified into several divisions on the basis of geographical and genetic origin, and on the position of the flowers. The species of this genus are taxonomically classified into seven sections (*Martagon, Pseudolirium, Lilium, Archelirion, Sinomartagon, Leucolirion and Oxypetalum*) (De Jong, 1974).

*Corresponding Author, Email: na.askari@yahoo.com

Propagation and micropropagation of lily

To maintain genetic purity, commercially grown cultivars are propagated by vegetative means. The natural vegetative propagules are small bulblets, either produced above ground on the stems (bulbils) or underground on bulb scales (Kumar et al., 2006). For conventional commercial vegetative propagation, bulblets are produced from scales. Excised scales are kept in a plastic bag in moistened vermiculite and each scale produces 1-4 bulblets. This procedure is called 'scaling' (Fig. 2). Because the speed is nonetheless relatively slow, introduction of newly bred cultivars still requires a long period of time (Langens-Gerrits and De Klerk, 1999).

Micropropagation has the potency to

produce large numbers of high-quality plantlets in a short period of time (George et al., 2008). Basically, micropropagation in lily is just like scaling but is carried out *in vitro* on an artificial nutrient medium. The major advantages of micropropagation are that small scale-explants can be used, that scales excised from the new bulblets can be used as new starting material so that per year a few propagation cycles can be performed, and that infection by micro-organisms is avoided (Fig. 3).

Fig. 1. *Lilium cvs* Santander (left) and Stargazer (right).

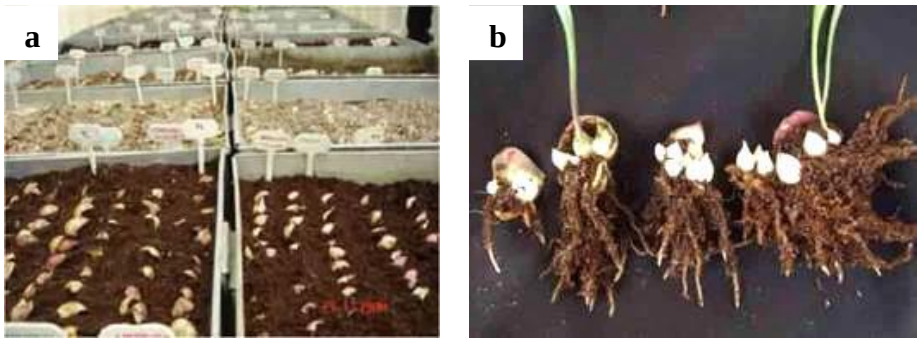

Fig. 2. Propagation of lily via scaling; a) lily scale culture on peat, b) regenerated lily bulblets after 12 weeks

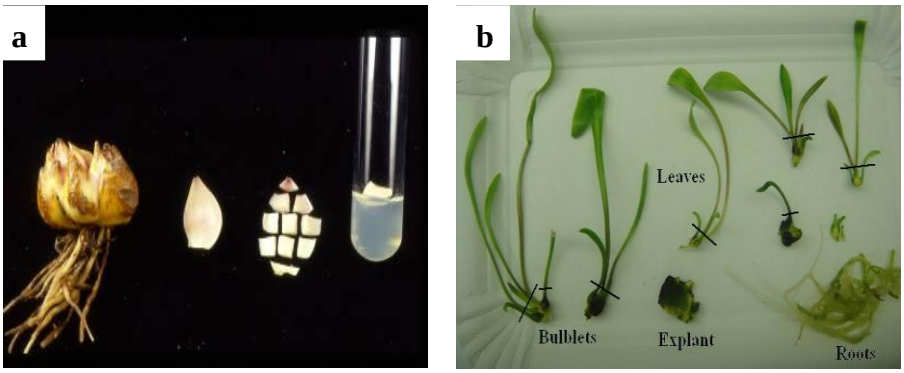

Fig. 3. Lily micropropagation *in vitro.* a) lily bulb, scale and explants cultured *in vitro;* b) lily bulblet regenerated *in vitro* after 12 weeks (bulblets, leaves, roots and scale explant).

The drawbacks of micropropagation are the high costs per propagule and the small size of the produced propagules. The latter leads to suboptimal performance after planting in soil. In micropropagation of *Lilium*, many tissues can be used but bulb scales are the favorite explants (Van Aartrijk and Van der Linde, 1986; Bahr and Compton, 2004; Han et al., 2005). Scales of lily bulbs are swollen petioles. Lily scale fragments cultured *in vitro* regenerate bulblets consisting of scales that may or may not carry a leaf blade (Jásik and De Klerk, 2006).

Importance of bulb formation in tissue culture

Bulblets and other storage organs produced *in vitro* have properties that make them preferable propagules. They can be easily handled, transported and stored and they do not require an extensive acclimatization procedure after transfer to soil (Thakur et al., 2006). In food crops, tissue culture is being used for production of microtubers in potato (Vreugdenhil et al., 1994), bulblets in shallot (Le Guen-Le Saos et al., 2002), garlic (Ravnikar et al., 1993), and onion (Knypl, 1980; Keller, 1993), and tuberous roots in sweet potato (Wang et al., 2006). In ornamentals, bulblets are produced as a last step during micropropagation of tulip (Kuijpers and Langens-Gerrits, 1996), lily (Bahr and Compton, 2004), *Narcissus* (Staikidou et al., 2005), *Hyacinthus* (Takayama et al., 1991), *Muscari* (Saniewski and Puchalski, 1987), *Hippeastrum* (Ilczuk et al., 2005) and iris (Van der Linde and Schipper, 1992). In some bulbous crops (lily and hyacinth), bulblets are generated 'automatically' under normal tissue culture conditions and no special measures have to be taken, but in other bulbous crops (tulip and iris) only shoots are formed. In the latter case, a special treatment is used to achieve bulb formation from the shoots.

The size of the bulblets produced *in vitro* has a strong effect on performance after planting. Studies with direct field planting of bulblets produced *in vitro* have shown that small bulblets emerge slower, less uniform and to a smaller percentage (Lian et al., 2003). Furthermore, the growth after planting is determined by the initial bulb weight. The initial weight influences growth after planting in two ways. The weight of bulblets after a growing season is linearly related with the initial weight (Langens-Gerrits et al., 1996). In addition, when bulblets are sufficiently large (> 300 mg), they increasingly sprout with a stem instead of a rosette (Langens-Gerrits et al., 2003a). When sprouting with a stem, growth of the bulblets in soil is much better (Fig. 4). The change from sprouting with a rosette to sprouting with a stem is related to a switch in ontogenetic development from juvenile to adult vegetative.

Fig. 4. Lily bulblet growth in soil (left: small bulblet growth without stem, right: bigger bulblet growth with a stem).

Effect of plant growth regulators on the growth of storage organs

All classes of plant growth regulators were found to have some effect on the formation of tubers and bulbs (Vreugdenhil and Sergeeva, 1999). The major ones are discussed below.

Gibberellins

Gibberellins inhibit tuberization, cause stolons to elongate rather than to swell, and inhibit starch accumulation and the synthesis of tuber-specific proteins in potato (Xu et al., 1998; Vreugdenhil and Sergeeva, 1999). The effect of the gibberellin-synthesis inhibitors on growth and development of oriental lily hybrids has been examined and with these growth retardants heavy bulblets were obtained (Kumar et al., 2005). The effect of growth retardants (like paclobutrazol or ancymidol) was studied in *Lilium* plantlets growing in liquid culture. A significant increase in leaf chlorophyll, epicuticular wax, plant dry weight and bulb starch contents were found in plantlets treated with growth retardants. A similar increase in the number of leaves, roots and bulbs was also noted (Thakur et al., 2006). Inhibitors of gibberellin biosynthesis (ancymidol, flurpirimidol, and paclobutrazol) promoted bulb formation and the percentage of bulbing in shallot (Le Guen-Le Saos et al., 2002). In *Hippeastrum* tissue culture, flurpirimidol not only influenced the propagation rate, but also the size of the newly developed bulblets. Explants cultured in media containing flurprimidol formed much bigger bulblets (Ilczuk et al., 2005).

Jasmonates (JA)

Exogenously applied JA induces or promotes tuber formation in potato, yam, and orchid, as well as bulb formation in garlic and narcissus. The putative role of JA in storage organ formation has been corroborated by reports on increased endogenous levels of JA in bulb and tuber forming plants (Jásik and De Klerk, 2006). In lily, JA promotes relative bulb weight (bulb weight as a percentage of plantlet weight). Here it should be noted again that scales of lily bulblets regenerated *in vitro* may or may not carry a leaf blade. In lily, JA strongly inhibited leaf blade formation without promoting absolute bulblet weight. JA significantly enhanced shoot and bulb development in garlic (Ravnikar et al., 1993). In *Narcissus triandus*, JA plays an important role in the formation and enlargement of bulbs (Santos and Salema, 2000).

Abscisic acid (ABA)

In lily, ABA promotes relative bulb weight (bulb weight as a percent of plantlet weight), but not the absolute weight: ABA strongly inhibits leaf blade formation without promoting absolute bulblet weight. When fluridone, an inhibitor of ABA synthesis is added, scale formation is inhibited completely, but is restored when ABA is added along with fluridone (Kim et al., 1994).

Effect of other factors on the growth of storage organs

Temperature

Both a moderate low temperature (15˚C) and a severe cold treatment (5˚C) have a profound effect on bulblet formation. A moderate temperature (15˚C) is required for a phase change. During the development of lily, three ontogenic phases can be distinguished, *viz*., juvenile, vegetative adult and flowering phases (Langens-Gerrits et al., 2003a). In *in vitro*-cultured lily bulblets, the transition from juvenile to vegetative adult is characterized by the development of a tunica-corpus structure with increased mitotic activity in the apical meristem, followed by stem elongation (Langens-Gerrits et al., 1996a). This step is related to the weight of the bulblets (Matsuo and Arisumi, 1978; Niimi, 1995; Langens-Gerrits et al., 2003a), and also to a moderate low temperature treatment (15˚C) (Ishimori et al., 2007).

In lily bulblets that are planted in the field, bulblet growth per mg leaf DW (this is the sink activity of the bulblets) is sharply promoted by a preceding cold treatment of bulblets at 5˚C (De Klerk, 2009). It was concluded that a cold treatment is necessary to stimulate sink strength of the bulblets. In tissue culture of lily, bulb formation occurs without a preceding cold treatment. Apparently other bulb-inducers (*e.g*., the high sucrose concentration in the medium) are sufficient. On the other hand, bulb growth *in vitro* is much less than bulb growth in soil (see below) and this may well be caused by the lack of cold treatment. In tulip, a cold treatment is also necessary to obtain bulblet growth (Rice et al., 1983).

Light

Light intensity and quality also influence the growth of bulblets. The fresh weight of bulblets was significantly greater in dark than in light in *L. longiflorum* (Leshem et al., 1982). In potato, darkening of both roots and shoots strongly promoted tuber formation; the tubers were formed on the darkened part of the plant (Aksenova et al., 1994). The influence of varying light treatments (blue, green, yellow, red, far-red and UV irradiation) on shoot and bulb induction was studied in tissue culture of hyacinths *in vitro*. Blue light stimulated growth and development of adventitious shoots and buds regardless of carbohydrate type, while the highest number of bulbs was obtained under red or white lights (Bach and Świderski, 2000).When stem cuttings of potato plants were cultured under red or blue lights, red light-grown plants were thin, long, with very small leaves, and produced no or only few microtubers (after longer-lasting cultivation). Blue light-grown plants remained short, thick, with large, well developed leaves and produced a significant amount of microtubers (Aksenova et al., 1994). In *L. ancifolium*, 16 h d^{-1} darkness had the best effect with respect to bulblet formation and enlargement (Zhang et al., 2010). In lily, deep red and deep red plus blue improved lily bulblet growth *in vitro* by 20% and 12% respectively compared with standard light conditions (Askari and De Klerk, 2018).

Explant size

In lily, the size of the explant (= scale fragment) has a major effect on the size of the regenerated bulblet. The size of the explant has yet another effect: bulblets of the same size that have regenerated from a large or a small explant respectively may differ with respect to the ontogenic age. The bulblets that have regenerated from a large explant are often adult and the ones from a small explant usually juvenile, and they sprout with a stem or with a rosette respectively (Langens-Gerrits et al., 2003a). There are a number of ways how the size can influence the regenerating bulblet, including hormonal and nutritional influences. Furthermore, more efficient scale explant sterilization surprisingly improved lily bulblet growth (Askari, et al. 2014, Askari and De Klerk, 2012). This could indicate that endogenous micro-organisms play a role in determining actual bulblet growth.

Carbohydrates

Sucrose does not affect the number of regenerated bulblets, but the size of bulblets increases with increasing concentrations of sucrose (Han et al., 2005). During *in vitro* culture, growth of the bulblets depends on the sucrose concentration (Yamagishi, 1998). It was reported earlier that large bulblets were obtained *in vitro* on medium with a high concentration of sucrose (Van Aartrijk and Van der Linde, 1986; Langens-Gerrits et al., 2003b). The increase of bulblet size with high concentrations of sucrose (60-90 gl^{-1}) was reported in many *Lilium* cultivars using different explants (Takayama and Misawa, 1982; Bonnier and Van Tuyl, 1997). The increase in bulblet size was mainly due to an increase in starch and total carbohydrates. The ontogenic age

of the regenerated bulblets is influenced by the sucrose-minerals ratio in the medium with a high ratio being promotive for the phase change. Especially phosphorus seems to be important but this has not been examined in detail (Langens-Gerrits et al., 2003a).

Lilium bulbs accumulate storage polysaccharides. Starch is the major storage polysaccharide in *Lilium* bulbs. In *L. longiflorum*, 85% of the storage polysaccharides are starch and the remainder glucomannan (Matsuo and Mizuno, 1974). Starch is an important reserve carbohydrate found in many plant species in all types of storage organs: seeds, tubers, bulbs and corms. It is deposited as crystalline a granule, that consists of two polysaccharides, amylose (20-30%) and amylopectin (70-80%). In plant storage organs, starch biosynthesis takes place within the amyloplast (Ji et al., 2003). With respect to the biochemistry, it is widely accepted that plastidic ADP-glucosepyrophosphorylase (AGPase) catalyzes the first step. AGPase utilizes glucose-1-phosphate (Glc-1-P) and ATP to form ADP-glucose (the substrate for starch synthase) and PPi, which serves as the direct precursor for starch synthesis. To date, the AGPase enzyme has been extensively studied in many sink organs of plants (Jaleel et al., 2007; Kato et al., 2007; Mohapatra et al., 2009). In mungbean seeds, a steady sink activity of the enzymes controlling carbon flux entering the seed may be required to achieve a large seed size (Chopra et al., 2007).

Abiotic Stress

Under stressful conditions, plants tend to increase allocation to below-ground biomass and storage organs (Puijalon et al., 2008). In correspondence with this, a short period of abiotic stress (heat, cold, anaerobiosis) increases rhizome growth in *Alstroemeria* (by ca. 100%, Pumisutapon et al., 2012), tuberization in potato (Pumisutapon et al., 2017) and bulblet growth in lily (Askari et al., 2016). In addition, moderate stress increases the growth of lily bulblets probably due to a protective mechanism against abiotic stress. This method is a useful way to stimulate lily bulblet growth *in vitro* to achieve bigger bulblets during tissue culture period. The optimal conditions as assessed in lily are anareobiosis and HAT (hot air treatment) to increase the growth of lily bulblets. A pre-treatment with moderate HAT stress (38°C) seems to be a valuable way to enhance the response to more severe HAT stress (47°C) (Askari et al., 2016).

Nutrition of the regenerating bulblet: how do medium components reach the regenerating bulblet?

The conditions in tissue culture seem to be optimal: water is abundantly available, high levels of organic and inorganic nutrients have been added and the temperature is favorable. Nevertheless, the growth of plantlets in tissue culture falls short of expectations and seems at best similar to growth in the field. This has been ascribed to poor long-distance translocation in the explants (De Klerk, 2010). Lily bulblets generated *in vitro* also show relatively poor growth. Growth in the tissue culture environment is linear and in the *ex vitro* condition exponential, resulting in much heavier bulblets (De Klerk et al., 1992). It should be noted that the *ex vitro* conditions include an adequate, constant temperature and relatively poor illuminance and/or lighting. There may be several reasons for the poor growth of bulblets in tissue culture:

1. The sink activity is limited; in tissue culture of lily no cold treatment is applied in contrast to, *e.g.*, tulip. In the field, a cold treatment increases sink activity in lily (De Klerk, 2009).
2. The bulblet requires hormonal factors for proper growth (ABA? JA? anti-gibberellins?), which are only available in complete plantlets growing in soil.

3. The tissue culture microenvironment is stressful, *e.g.*, because sucrose is toxic (Desjardins et al., 2009).
4. The supply of nutrients is inadequate.

The latter factor will be elaborated below. First, the supply under natural conditions will be discussed.

Translocation of inorganic nutrients and carbohydrates under natural conditions

In plants growing in the field, inorganic nutrients are taken up from the soil by the roots. Movement of solutes from the soil into the cell walls of roots occurs by diffusion or by 'hitching' with the mass water flow. The ions move in the apoplast and the symplast up to the xylem parenchyma cells. However, a suberized cell layer in the endodermis, known as the Casparian strip, effectively blocks the entry of water and mineral ions into the stele via the apoplast. To pass through this cell layer, the solutes have to move into the symplast. After passing the Casperian strip, they may again move both in the symplast and the apoplast. The Casperian strip also prevents moving backwards via the apoplast. Next, the solutes are loaded into the xylem tracheary cells and are then taken with the water flow in the xylem to the shoot.

In plants growing in the field there are two main sources of carbohydrates: photosynthetically active leaves and degrading storage organs. Sucrose is the major transport mode for carbohydrates and long-distance transport from source to sink organs occurs in the phloem (Li et al., 2010). In the source tissue, energy is necessary to move carbohydrates from producing cells into the sieve elements in the phloem. This movement is called phloem loading. In the sink tissue, energy is essential for some aspects of movement from sieve elements to sink cells, which store or metabolize the sugars. This movement of photosynthate from sieve elements to sink cells is called phloem unloading (Taiz and Zeiger, 2002). Phloem functions differ according to organ location. At least three parts can be defined: collection phloem in source organs (minor veins), transport phloem (along the path from source to sinks) and release phloem in sink organs (Van Bel, 1993). Sink organs rely heavily on the delivery of carbohydrates through the phloem for growth and development. Besides sucrose, other sugars are found and sometimes may be as abundant as sucrose, depending on species. They include polyols and oligosaccharides of the raffinose family. Other nutrients, such as amino acids and organic acids, are also found (Zimmermann and Ziegler, 1975). The loading of sucrose at the source and unloading at the sink brings about differences in osmotic potential that lead to uptake of water in the source and release in the sink and thereby the water flow in the phloem from source to sink.

Transport to regenerating bulblets cultured in vitro

The components that are being translocated into growing bulblets include carbohydrates and inorganic nutrients. Carbohydrates originate from the scale explant and from the medium (Langens-Gerrits et al., 2003b). The percentage bulb growth that can be attributed to uptake of medium-sucrose is constant over the full regeneration period: 45-50 % for large (3 x 15 mm) and 65-75 % for small (3 x 5 mm) explants.

It is usually taken for granted that medium components reach the target tissue in the explants (usually the growing areas) in adequate amounts. But how are they actually translocated? Generally, solutes (compounds dissolved in water) may be translocated in two ways: (1) by diffusion and (2) by hitch-hiking with the water flow. Diffusion is driven by random thermal agitation and is fast over short distance, but very slow over large distances. According to Flick's law of diffusion, diffusion over 1 meter takes 32 years, over 2 cm one week, and over 50 μm

2.5 sec. Therefore, plants use the water flow in the vascular tissues for long distance transport (Taiz and Zeiger, 2002). It has been discussed in the previous section how compounds are translocated to growing areas in plants in the field. In micropropagation, the distances between the source, the medium, and the sink (the growing areas) are a few millimeters to a few centimeters. Diffusion over a distance of 4 mm takes about 9 h (depending on the diffusion-coefficient) and 2 cm takes 8 days which is slow. Moreover, these calculated speeds are in water without any obstacles. Hindrances such as membranes will slow down the movement in living tissues considerably. Furthermore, a third factor that results in slow translocation via diffusion is the small diameter of the tissue via which transport occurs. Therefore, to obtain adequate growth in tissue culture most of the long distance transport of nutrients (from the medium to the growing regions of the shoot or from the medium to the developing bulblets) should also occur via the vascular bundles (De Klerk, 2010).

Transport in xylem and phloem in vitro

The movement of water in the xylem is driven by transpiration. The tissue culture conditions are very humid so transpiration is expected to be reduced. Transpiration of shoots cultured *in vitro* has only been measured few times and was found to be 50 $\mu l.cm^{-2}.d^{-1}$ (Tanaka et al., 1991) and 30 $\mu l.cm^{-2}.d^{-1}$ (De Klerk, 2010). This is a small percentage of the transpiration rate in the field. Calculations showed that this may be just enough to support growth when the compounds enter the cut surface together with water and move together with the water upwards into the shoot. Experiments with dyes, though, showed that when the cut surface is healed after the wounding reaction, most of the dye stays in the tissue at the cut surface. Similarly inorganic and organic nutrients may be captured at the cut surface. Regenerating lily bulblets that have no leaves because of culture in the dark, will have even less transpiration and still show bulblet growth similar or even higher than light-grown bulblets. We therefore conclude provisionally that xylem transport does not play a major role in the growth of lily bulblets.

About phloem functioning in tissue culture nothing is known. As noted above, water flow in the phloem -so also the movement of solutes- is brought about by the loading and unloading of sucrose. In lily tissue culture, phloem unloading occurs in the regenerating bulblets and the mechanisms are most probably the same as in bulblets growing under natural conditions. Loading may occur in scale explants or in the leaves where sufficient sucrose might have accumulated by photosynthesis and/or by translocation in the transpiration flow in the xylem. In lily scale explants, the scale itself also functions naturally as a source when being degraded. It is not known in which developmental stage scales in tissue culture are. Scales seem to be both sink and source: They definitely act as sink during tissue culture since they increase in weight but they also act as source since a significant portion of the carbohydrates translocated to the regenerating bulblet is scale-carbohydrate (Langens-Gerrits et al., 2003b). In potato, ^{14}C-glucose is incorporated in starch when the potato is a sink and in sucrose when it is a source (Viola et al., 2007). It is questionable whether sufficient loading occurs in the regenerating bulblet itself, so the scale explant seems to play a major role. It should be remembered that -in agreement with this- the size of the scale explant determined the size of the regenerating bulblet (Langens-Gerrits et al., 2003b). In addition, the size and the number of vascular bundles in scale explants has a strong effect on lily bulblet growth *in vitro* (Askari, 2016).

Conclusions and future prospects

In all geophytes, also in the economically prominent ornamental geophytes like tulip, lily, hyacinths, iris and narcissuses, the formation of storage organs *in vitro* is a slow and problematic process. Although micropropagation protocols have been developed for all major geophytes, most are not sufficiently workable in commercial laboratories and a major reason for this is inadequate bulb formation. So research is needed to study and improve *in vitro* storage organ formation.

References

1. Aksenova N.P, Konstantinova T.N, Sergeeva L.I, Macháčková I, Golyanovskaya S.A. 1994. Morphogenesis of potato plants in vitro. I. Effect of light quality and hormones. Journal of Plant Growth Regulation 13, 143-146.
2. Askari N. 2016. Aspects of bulblet growth of lily in vitro. Wageningen University PhD Thesis.130. DOI: 10.18174/375959
3. Askari N, De Klerk G.J. 2012. Avoidance of cross-contamination during the initiation step in lily tissue culture. The Lily Yearbook North American Lily Society 39-44.
4. Askari N, De Klerk G.J. 2018. The effect of light quality on lily growth in vitro. 2th International and 3rd National Ornamental Plant Congress. Iran.
5. Askari N, Visser R, De Klerk G.J. 2016. The role of abiotic stresses on lily bulblet growth in vitro. Propagation of Ornamental Plants 16, 130-136.
6. Askari N, Wang Y.G, De Klerk G.J. 2014. In tissue culture of Lilium explants may become heavily contaminated by the standard initiation procedure. Propagation of Ornamental Plants 14, 49-56.
7. Bach A, Świderski A. 2000. The effect of light quality on organogenesis of *Hyacinthus orientalis* L. *in vitro*. Acta Biologica Cracoviensia. Series Botanica 42, 115-120.
8. Bahr L.R, Compton M.E. 2004. Competence for *in vitro* bulblet regeneration among eight *Lilium* genotypes. HortScience 39, 127-129.
9. Beattie D, White J. 1993. *Lilium*-hybrids and species. In: The Physiology of Flower Bulbs. A. De Hertogh and M. Le Nard (eds) Elsevier Science Publishers.
10. Bonnier F, Van Tuyl J. 1997. Long term *in vitro* storage of lily: effects of temperature and concentration of nutrients and sucrose. Plant Cell, Tissue and Organ Culture 49, 81-87.
11. Chopra J, Kaur N, Gupta A.K. 2007. Sustained activities of carbon metabolizing enzymes determine seed size in *Vigna radiata* (mungbean). Current Science-Bangalore 92, 1420-1424.
12. De Jong P. 1974. Some notes on the evolution of lilies. The Lily Yearbook of the North American Lily Society.
13. De Klerk G.J, Kim K, Van Schadewijk M, Gerrits M. 1992. Growth of bulblets of *Lilium speciosum in vitro* and in soil. Acta Horticulturae 325, 513-520.
14. Desjardins Y, Dubuc J, Badr A. 2009. *In vitro* culture of plants: a stressful activity. Acta Horticulturae 812, 29-50.
15. George E.F, Hall M.A, De Klerk G.J. 2008. Plant Propagation by Tissue Culture: Volume 1. The Background. Springer 520.
16. Han B.H, Yae B.W, Yu H.J, Peak K.Y. 2005. Improvement of *in vitro* micropropagation of *Lilium oriental* hybrid 'Casa blanca'by the formation of shoots with abnormally swollen basal plates. Scientia Horticulturae 103, 351-359.
17. Ilczuk A, Winkelmann T, Richartz S, Witomska M, Serek M. 2005. *In vitro* propagation of *Hippeastrum* × *chmielii* Chm. influence of flurprimidol and the culture in solid or liquid medium and in temporary immersion systems. Plant Cell, Tissue and Organ Culture 83, 339-346.
18. Ishimori T, Niimi Y, Han D.S. 2007. Benzyladenine and low temperature promote phase transition from juvenile to vegetative adult in bulblets of *Lilium*× *formolongi* 'White Aga'cultured *in vitro*. Plant Cell,Tissue and Organ Culture 88, 313-318.
19. Jásik J, De Klerk G.J. 2006. Effect of methyl jasmonate on morphology and dormancy development in lily bulblets regenerated *in vitro*. Journal of Plant Growth Regulation 25, 45-51.
20. Ji Q, Vincken J.P, Suurs L.C, Visser R.G. 2003. Microbial starch-binding domains as a tool for targeting proteins to granules during starch biosynthesis. Plant Molecular Biology 51, 789-801.
21. Kato T, Shinmura D, Taniguchi A. 2007. Activities of enzymes for sucrose-starch

conversion in developing endosperm of rice and their association with grain filling in extra-heavy panicle types. Plant Production Science 10, 442-450.

22. Keller E. 1993. Sucrose, cytokinin, and ethylene influence formation of *in vitro* bulblets in onion and leek. Genetic Resources and Crop Evolution 40, 113-120.

23. Kim K.S, Davelaar E, De Klerk G.J. 1994. Abscisic acid controls dormancy development and bulb formation in lily plantlets regenerated *in vitro*. Physiologia Plantarum 90, 59-64.

24. Knypl J. 1980. Stimulation of bulb growth in onion (*Allium cepa* L.) by N, N-diethyl-N-(2-hydroxyethyl) glycine. Biologia Plantarum 22, 226-230.

25. Kuijpers A.M, Langens-Gerrits M.M. 1996. Propagation of tulip *in vitro*. Acta Horticulturae 430, 321-324.

26. Kumar S, Kanwar J, Sharma D. 2006. *In vitro* propagation of *Lilium*. Advances in Horticultural Science 2, 181-188.

27. Kumar S, Kashyap M, Sharma D. 2005. *In vitro* regeneration and bulblet growth from lily bulb scale explants as affected by retardants, sucrose and irradiance. Biologia Plantarum 49, 629-632.

28. Langens-Gerrits M.M, De Klerk G.J. 1999. Micropropagation of flower bulbs. In: Plant Cell Culture Protocols, Springer, pp. 141-147.

29. Langens-Gerrits M.M, De Klerk G.J, Croes A. 2003a. Phase change in lily bulblets regenerated *in vitro*. Physiologia Plantarum 119, 590-597.

30. Langens-Gerrits M.M, Kuijpers A.M, De Klerk G.J, Croes A. 2003b. Contribution of explant carbohydrate reserves and sucrose in the medium to bulb growth of lily regenerated on scale segments *in vitro*. Physiologia Plantarum 117, 245-255.

31. Langens-Gerrits M.M, Lilien-Kipnis H, Croes T, Miller W.B, Kollöffel C, De Klerk G.J. (1996). Bulb growth in lily regenerated *in vitro*. Acta Horticulturae 430, 267-274.

32. Le Guen-Le Saos F, Hourmant A, Esnault F, Chauvin J. 2002. *In vitro* bulb development in shallot (*Allium cepa* L. Aggregatum Group): effects of anti-gibberellins, sucrose and light. Annals of Botany 89, 419-425.

33. Leshem B, Lilien-Kipnis H, Steinitz B. 1982. The effect of light and of explant orientation on the regeneration and subsequent growth of bulblets on *Lilium longiflorum* Thunb, bulb-scale sections cultured *in vitro*. Scientia Horticulturae 17, 129-136.

34. Matsuo E, Arisumi K. 1978. Studies on the leaf development of the scale bulblet in the Easter lily (Lilium longiflorum). III. The relationship between the polarity of a scale and the leaf emergence of the scale bulblet or the plant type. Journal of the Japanese Society for Horticultural Science 47(3): 415-420

35. Matsuo T, Mizuno T. 1974. Changes in the amounts of two kinds of reserve glucose-containing polysaccharides during germination of the Easter lily bulb. Plant and Cell Physiology 15, 555-558.

36. Mohapatra P, Sarkar R, Kuanar S. 2009. Starch synthesizing enzymes and sink strength of grains of contrasting rice cultivars. Plant Science 176, 256-263.

37. Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15, 473-497.

38. Niimi Y. 1995. *In vitro* propagation and post-*in vitro* establishment of bulblets of *Lilium japonicum* Thunb. Journal of the Japanese Society for Horticultural Science 63, 843-852.

39. Puijalon S, Piola F, Bornette G. 2008. Abiotic stresses increase plant regeneration ability. Evolutionary Ecology 22, 493-506.

40. Pumisutapon P, Topoonyanont N. 2017. Moderate-abiotic stress increase in vitro tuberization and microtuber growth of potato. Acta Horticulturae 1155.

41. Pumisutapon P, Visser R.G, De Klerk G.J. 2012. Moderate abiotic stresses increase rhizome growth and outgrowth of axillary buds in Alstroemeria cultured in vitro. Plant Cell, Tissue and Organ Culture 110, 395-400.

42. Ravnikar M, Žel J, Plaper I, Špacapan A. 1993. Jasmonic acid stimulates shoot and bulb formation of garlic *in vitro*. Journal of Plant Growth Regulation 12, 73-77.

43. Rice R, Alderson P, Wright N. 1983. Induction of bulbing of tulip shoots *in vitro*. Scientia Horticulturae 20, 377-390.

44. Saniewski M, Puchalski J. 1987. The effect of methyl jasmonate and abscisic acid on differentiation of benzyladenine-induced bulblets in *Muscari* bulbs. Biologia Plantarum 29, 63-65.

45. Santos I, Salema R. 2000. Promotion by

Jasmonic acid of bulb formation in shoot cultures of *Narcissus triandrus* L. Plant Growth Regulation 30, 133-138.

46. Staikidou I, Watson S, Harvey B.M, Selby C. 2005. Narcissus bulblet formation *in vitro*: effects of carbohydrate type and osmolarity of the culture medium. Plant Cell, Tissue and Organ Culture 80, 313-320.
47. Taiz L, Zeiger E. 2002. Plant Physiology. 3rd edition. New York, Sinauer, 690.
48. Takayama S, Amo T, Fukano M. 1991. Rapid clonal propagation of *Hyacinthus orientalis* bulbs by shake culture. Scientia Horticulturae 45, 315-321.
49. Takayama S, Misawa M. 1982. Regulation of organ formation by cytokinin and auxin in *Lilium* bulbscales grown *in vitro*. Plant and Cell Physiology 23, 67-74.
50. Tanaka A, Hoshi Y, Kondo K, Taniguchi K. 1991. Induction and rapid propagation of shoot primordia from shoot apices of *Lilium japonicum*. Plant Tissue Culture Letters 8, 206-208.
51. Thakur R, Sood A. 2006. An efficient method for explant sterilization for reduced contamination. Plant Cell, Tissue and Organ Culture 84, 369-371.
52. Van Aartrijk J, Van der Linde P. 1986. *In vitro* propagation of flower-bulb crops. In: Tissue culture as a plant production system for horticultural crops, Springer 317-331.
53. Van Bel A. 1993. Strategies of phloem loading. Annual Review of Plant Biology 44, 253-281.
54. Van der Linde P, Schipper J. 1992. Micropropagation of iris with special reference to *Iris x hollandica* Tub. In: Biotechnology in Agriculture and Forestry 20, 173-197.
55. Viola R, Pelloux J, Van Der Ploeg A, Gillespie T, Marquis N, Roberts A.G, Hancock R.D. 2007. Symplastic connection is required for bud outgrowth following dormancy in potato (*Solanum tuberosum* L.) tubers. Plant, Cell & Environment 30, 973-983.
56. Vreugdenhil D, Sergeeva L.I. 1999. Gibberellins and tuberization in potato. Potato Research 42, 471-481.
57. Vreugdenhil D, Bindels P, Reinhoud P, Klocek J, Hendriks T. 1994. Use of the growth retardant tetcyclacis for potato tuber formation *in vitro*. Plant Growth Regulation 14, 257-265.
58. Wang Q.M, Zhang L.M, Guan Y.A, Wang Z.L. 2006. Endogenous hormone concentration in developing tuberous roots of different sweet potato genotypes. Agricultural Sciences in China 5, 919-927.
59. Xu X, van Lammeren A.A, Vermeer E, Vreugdenhil D. 1998. The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation *in vitro*. Plant Physiology 117, 575-584.
60. Zhang Y, Zhang Q, Xue X. 2010. The effects of the photoperiods on the bulblet formation and sugar metabolism change of wild *Lilium lancifoiium in vitro*. Acta Horticulturae Sinica 201, 957-962.
61. Zimmermann M.H, Ziegler H. 1975. List of sugars and sugar alcohols in sieve-tube exudates. Encyclopedia of Plant Physiology. Springer, New York 480-503.