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Differences in regenerative capacity of Oriental lily (*Lilium sp.*) cultivars

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ABSTRACT: The regenerative potential of *in vitro*-produced bulblets was investigated in three commercial Oriental lily cultivars ('Aubade', 'Belcanto' and 'Solaia') in relation to two synthetic cytokinins BA and TDZ and picloram as representative of auxins. Single bulblet leaflets were excised and cultured on MS medium supplemented with either 6-benzyladenine (BA 0-2.0 mg/l), thidiazurone (TDZ 0-2.0 mg/l) or picloram (PIC 0-3.0 mg/l). In all three cultivars and medium combinations explants after 5 weeks regenerated somatic embryos, bulblets and plantlets. While bulblet production was balanced, plantlet and somatic embryogenesis (SE) production were complementary with pronounced SE production at higher plant growth regulator concentrations and plantlet production at lower concentrations. Picloram had a sharp regeneration demarcation with low plantlet production above 0.5mg/l. BA and TDZ produced SE at all concentrations including hormone-free controls. On media with TDZ and BA there was a gradual change from bulblet regeneration at lower to somatic embryogenesis at higher concentrations. For all three cultivars, details of the regeneration process were studied by histological techniques in TDZ-supplemented medium, showing early stage SE regeneration in all samples. Mature, elongated SE stages were missing, indicating early transition of SE into bulblets. The optimal propagation conditions were elaborated for all three lily cultivars.

Key words: bulblets, somatic embryogenesis, regeneration, Oriental lilies, BA - 6-benzylamino purine, TDZ - thidiazurone (N-phenyl-N'-1,2,3-thiadiazol-5-yl urea), PIC – picloram

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INTRODUCTION

The genus *Lilium* comprises more than 90 bulb-bearing ornamental monocotyledonous species, some of which are mass propagated to be sold as cut flowers. The natural vegetative propagation of lilies from bulbs is slow and tedious; therefore employment of *in vitro* techniques provides a substantial improvement in the propagation rate and productivity. Lilies can be mass propagated from various explant types using different *in vitro* culture techniques..

First trials with the use of *in vitro* culture techniques were done by Rob (1957) and Sheridan (1968). Full techniques were soon elaborated by Simmonds & Cummings (1976), Stimart & Ascher (1978), Novak & Petru (1981), Van Aartrijk & Blom-Barnhoorn (1981), Takayama & Misawa (1982, 1983), Van Aartrijk *et al.* (1990), Wickremesinhe *et al.* (1994) and later many others.

The scope of explants used for *in vitro* regeneration of *Lilium* species ranges from bulblet scales (Niimi 1985; Bakhshaie *et al.* 2010), nodal segments (Kapoor *et al.* 2009), leaf pedicels (Liu & Burger 1986), callus tissues (Tribulato *et al.* 1997), petioles (Tang *et al.* 2010), leaves (Bacchetta *et al.* 2003; Kanchanapoom *et al.* 2011; El-Naggar *et al.* 2012), and roots (Kumar *et al.* 2008). However, it has been shown that bulblet scale explants have the highest potential for regeneration of new bulblets (Stimart & Ascher 1978; Takayama & Misawa 1983; Gerrits *et al.* 1992; Wickremesinhe *et al.* 1994).

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Induction of bulblets on leaf and bulb explants can be used for propagation of endangered Lilium species as in the case of *Lilium bosniacum* (Parić *et al.* 2011)

From the late 1970s and early 1990s it was expected that the fast progress in plant *in vitro* culture studies would eventually result in detection of novel plant growth regulators which could promote the shoot regeneration in monocotyledonous species in a way similar to the action of cytokinins in dicotyledonous species. This did not happen, though for many years investigators were evaluating the effects of novel plant growth regulators like dicamba, thidiazurone, picloram, various growth retardants and many other compounds. Some of them, like thidiazurone or 2iP, even entered common usage providing valuable aids in certain situations, but none was able to trigger shoot regeneration comparable to the process observable in dicotyledonous species.

Thus micropropagation, which in dicotyledonous species is based on the activation of axillary buds located at leaf axils, in monocotyledonous species is usually replaced by regeneration of adventitious buds or somatic embryogenesis. Luckily, monocotyledonous species have increased genome stability usually providing true-to-type offspring plantlets even after regeneration from undifferentiated tissues.

The change in the body structure of bulbous species including lilies is a case of extreme positive adaptation. These plants through all of their life cycle rigorously prevent shoot elongation which occurs only when the plant is ready for flowering. This process, known as bolting, induces fast elongation of flowering stalks and is at least partly controlled by plant growth regulators with gibberellic acids playing the major role. Flowering of bulb-bearing ornamental plants can be fine-tuned by controlling light intensity, photoperiod duration and temperature during storage.

With a strict regulation of shoot elongation it is not surprising that in lilies adventitious shoot buds following regeneration have conserved inhibition of shoot elongation imposing their development in the form of bulblets. Even axillary buds on nodal segments of *L. longiflorum* upon excision further developed as bulblets (Nhut 1998). However, the truly interesting features of regeneration in lilies are 1 - that a plant growth regulator can at the same time support two different regeneration processes (bulblet regeneration and somatic embryogenesis) in the same explants and 2 - that a regeneration process like somatic embryogenesis (SE) in the same tissue can be promoted by different groups of plant growth regulators (auxins, cytokinins, retardants).

The first study of SE in bulb scale explants using 23 cultivars of Oriental lilies was done by Haensch (1996). SE was genotype-dependent as it occurred only in 4 cultivars on 2,4-D or picloram-supplemented media. Auxins were also used as SE inducers in studies of Tribulato *et al.* (1997) and Pelkonen and Kauppi (1999). Picloram at 3 mg/l was highly efficient in inducing SE in Oriental hybrids (Kim *et al.* 2003). Cytokinins were shown to affect SE in studies of Nhut *et al.* (2006), Khosravi *et al.* (2007) and Bakhshaie *et al.* (2010). Finally, plant growth retardants (paclobutrazol) were also shown by Kumar *et al.* (2005) to affect and induce SE in lilies. The role of plant growth regulators in SE has been reviewed by Jiménez (2005).

In this study we analyzed the ability of bulblet-scale explants to simultaneously regenerate adventitious buds and somatic embryogenesis on media supplemented with cytokinins BA and TDZ, or picloram. The distinction between SE and adventitious bud regeneration was made by histological studies of cultured samples. Extended culture of regenerated bulblets was also followed and for each cultivar the optimal production conditions for extended bulblet multiplication was formulated.

MATERIAL AND METHODS

Induction of morphogenesis . The starting material was bulbscale leaflets of Oriental lilies excised from bulblets of cultivars 'Aubade', 'Belcanto' and 'Solaia', previously regenerated on MS medium (Murashige & Skoog 1962) with 0.1 mg/l BA (6-benzyl adenine) and 0.1 mg/l NAA (naphthylacetic acid). Media used to trigger regeneration had 3% sucrose, 0.7% agar and 100 mg/l myo-inositol, supplemented with either picloram (PIC at 0, 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 mg/l), thidiazurone (TDZ at 0, 0.1, 0.2, 0.5, 1.0 or 2.0 mg/l) or benzyl adenine (BA at 0, 0.1, 0.2, 0.5, 1.0 or 2.0 mg/l). Medium pH was adjusted to 5.8 prior to autoclaving at 114^{0}C for 25 min. Explants (10-15) were cultured in Petri plates in a growth room adjusted to 25±2°C and a photoperiod of 16/8 h light/dark (long day). Cool white fluorescent lamps (Philips) provided 35-45 μM m^{-2} s^{-1} irradiance at the level of cultures. Subculture duration was fixed at five weeks. All treatments were replicated three times.

Bulblet development. To evaluate the development of freshly regenerated bulblets they were further cultured individually in 150 ml flasks with 50 ml MS basal medium (3% sucrose, 0.7% agar and 100 mg/l myo-inositol) with the addition of 0.05–0.5 mg/l BA and 0.1 mg/l NAA. Growth parameters scored after 8 weeks were length of the first leaf, number of new bulblets, percentage of rooted cultures, number of roots per culture, length of the longest root, final bulblet diameter (mm) and fresh mass. Treatments were replicated three times.

Histological analysis of somatic embryogenesis. Material for histological analysis of somatic embryogenesis was sampled after 5 weeks of growth on TDZ-supplemented media. Samples were fixed in formalin:glacial acetic acid:70% ethanol at 10:5:85 v/v for 48 h, and further

processed by embedding in Histowax (HistoLab, Sweden). Blocks sectioned at 7 μm thickness were stained with haematoxylin (Jensen 1962) and photographed under a Leitz DMRB photomicroscope (Leica, Wetzlar, Germany).

Statistical analysis. Data were analyzed by ANOVA using Statgraph 2.1 (Statistical Graphics Corporation, USA). Results are presented as means with standard errors. Significance of differences between treatments is shown by different letters following the means according to Fisher's multiple range test (LSD) with a significance level of $P \leq 0.05$. In treatments with three categories of regenerants, significance is indicated separately for each group and capital Latin letters refer to plantlets, lower case Latin letters to bulblets, and Greek letters to early SE regenerants.

RESULTS

Regeneration types. The effect of picloram PIC (0.5-3.0 mg/l) on the regeneration of bulblet explants is presented in Fig. 1 and Fig. 4a,b,d,e,g,h. In all three cultivars, the major responses on PIC-supplemented media were bulblet production and early SE. Bulblet regeneration at 2–3 regenerants was stable at all PIC concentrations except at the highest concentrations in cv. 'Solaia' where it was lower. Early SE appeared at all concentrations and in all cultivars, though it alternated with the production of plantlets at lower concentrations. On hormone-free medium, early SE was absent and all cultivars had more than two plantlets regenerated per explant. At 0.5 mg/l cultivars simultaneously produced all three types of regenerants. In cv. 'Aubade' on media with 0.5-1.5 mg/l picloram SE regeneration was direct.

The highest production of SE in 'Aubade' and 'Solaia', (2.60 and 2.89 respectively) was on 0.5 mg/l PIC, while for cv. 'Belcanto' it was medium with 2.0 mg/l PIC producing 3.44 SE/explant (for small SE even 11.07). The formation of friable yellow-colored callus also increased with picloram concentration. Cultivars differed significantly with 95.3% being the highest frequency of callus, occurring in 'Belcanto', growing on 2.0 mg/l PIC.

On media supplemented with 0.1–2.0 mg/l TDZ, SE were directly regenerated in all three cultivars (Fig. 2, Fig. 4c,f,i). SE production was dominant in relation to the production of bulblets. Production of plantlets which was prominent on media with low TDZ concentrations alternated with SE regeneration which was clearly promoted by TDZ. All cultivars at all TDZ concentrations including the plant growth regulator-free medium produced the three types of regenerants.

Bulblets regenerated on all media types most frequently on hormone-free medium indicating that TDZ was unnecessary for bulblet regeneration, though TDZ inhibited rooting. In cultivars 'Belcanto' and 'Aubade' the highest number of SE was 4.7 and 3.2, respectively, on

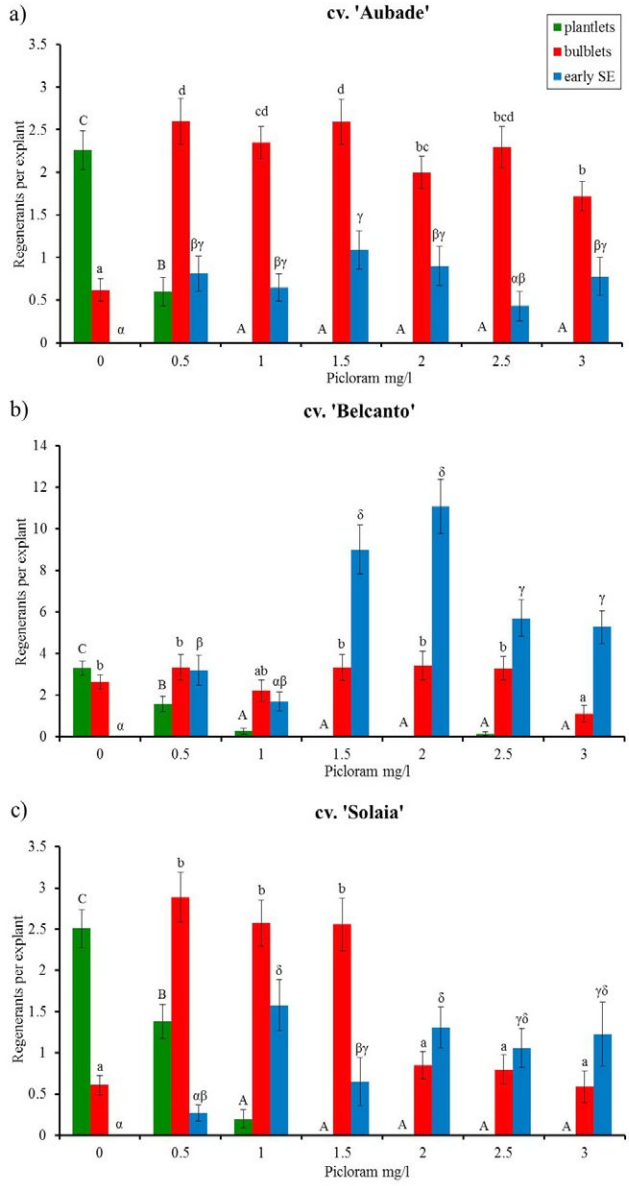

Fig 1. Regeneration of early SE, bulblets and plantlets in the bulblet leaf explants cultured at different Picloram concentrations. Columns show average values and standard error of the mean for each category of regenerant. Letters denote statistical significance with full details in M & M.

medium with 0.2 mg/l TDZ, while in 'Solaia' it was 3.5 but at a higher TDZ concentration (2.0 mg/l).

On BA-supplemented media (Fig. 3), the production of regenerants at all BA concentrations and in all cultivars closely followed the results obtained with TDZ (Fig. 2). However, the SE regeneration induced by BA was always indirect. Explants first proliferated yellowish, friable callus from which SE later regenerated. Due to the interpolation of a callus stage, subsequent SE production differed and in all stages of development productivity was somewhat lower than in TDZ-induced SE. At the highest BA concentration (2.0 mg/l), callusing occurred in around

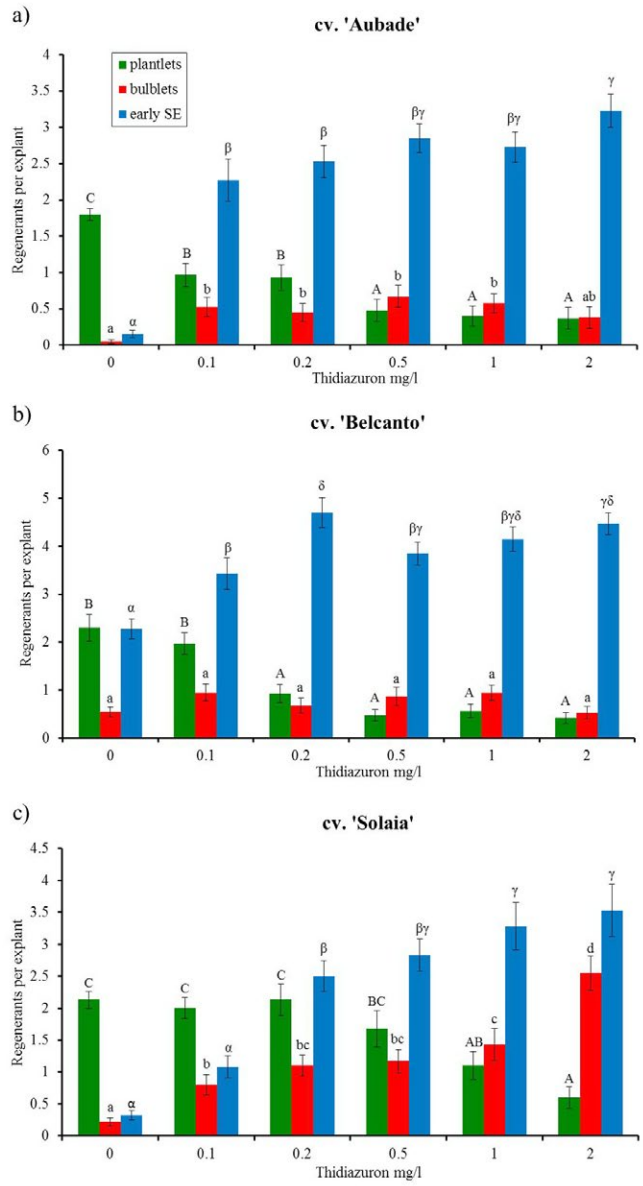

Fig 2. Regeneration of early SE, bulblets and plantlets in the bulblet leaf explants cultured at different Thidiazurone concentrations. Columns show average values and standard error of the mean for each category of regenerant. Letters denote statistical significance with full details in M & M.

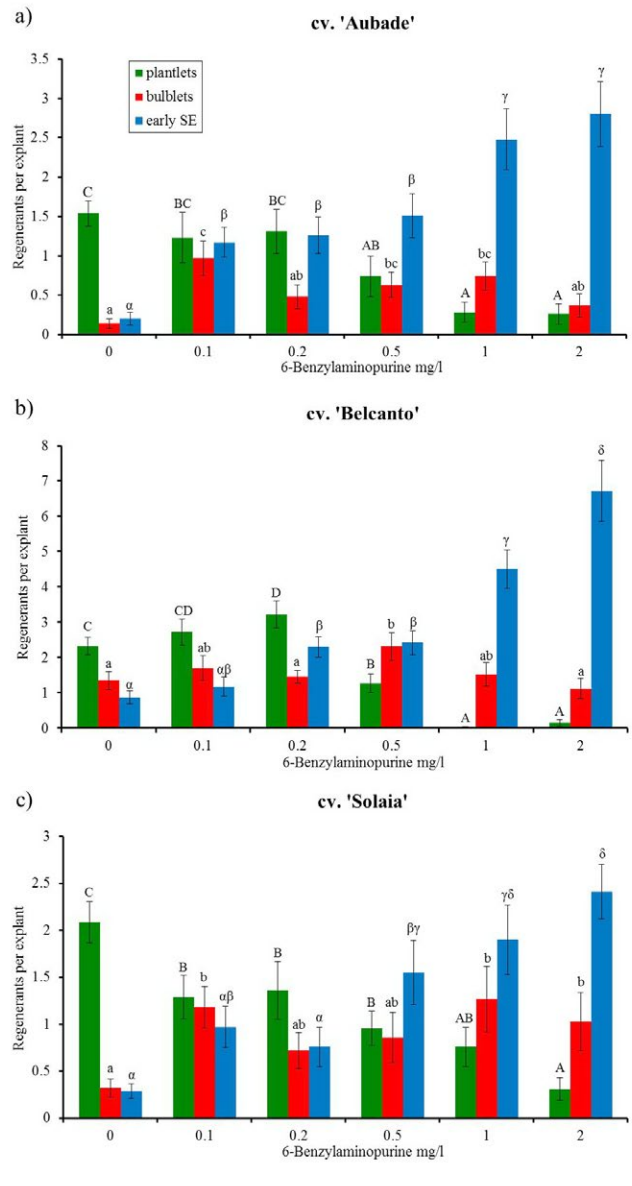

Fig 3. Regeneration of early SE, bulblets and plantlets in the bulblet leaf explants cultured at different BA concentrations. Columns show average values and standard error of the mean for each category of regenerant. Letters denote statistical significance with full details in M & M.

50% of explants of all cultivars and SE regenerated in 90% of explants proliferating callus. 'Belcanto', with average of 6.7 of SE per explant, produced nearly twice the SE of 'Solaia' and 'Aubade'. In 'Solaia', direct SE regeneration was also observed on media with 0.1-0.5 mg/l BA. The highest bulblet formation was registered on media with 0.2 mg/l BA in all cultivars.

Further development of regenerated bulblets. Development of bulblets regenerated on bulblet leaf explants was followed further on media supplemented with 0.05-0.5 mg/l BA and NAA fixed at 0.1 mg/l (Table 1). After 8 weeks, bulblet growth was scored for several parameters. In general, the original bulblets increased their size and weight developing into plants, though they also regenerated some new bulblets.

Increasing the BA content decreased the length of leaves, percentage of rooted cultures, average root number per explant and average root length in all cultivars (Fig. 5). BA also decreased the bulblet fresh weight but did not affect the diameter of bulblets.

Cv. 'Aubade' had the longest leaves (57.8 mm) and highest number of newly-formed bulblets (3.4) at 0.05 mg/l BA. The higest rooting (100%) was obtained in 'Aubade'

Table 1. Further development of bulblets regenerated on PIC, BA and TDZ induction media transferred to maintenance MS media supplemented with 0.1 mg/l NAA and 0.05 – 0.5 mg/l BA. Results were scored after 8 weeks with n=64 explants per treatment. ANOVA was done separately for every cultivar, different letters indicate significant developmental differences according to Fisher's multiple range test (LSD) at $P \leq 0.05$.

Cultivar	BA (mg/l)	Increase of bulblet diameter (mm) ± SE	% increase of fresh weight ± SE	Length of the first leaf (mm) ± SE	% regenerated bulblets	Production of new bulblets ± SE	Rooting %	Roots per bulblet ± SE	Length of the longest root (mm) ± SE
'Aubade'	0.05	3.5 ± 0.4 a	**665.6 ± 62.9 b**	**57.8 ± 2.3 b**	70.3	2.5 ± 0.3 a	**100**	**7.1 ± 0.5 c**	**34.6 ± 1.8 c**
	0.1	3.5 ± 0.3 ab	573.7 ± 62.2 ab	53.4 ± 2.2 b	73.4	2.7 ± 0.3 ab	93.7	4.7 ± 0.3 b	24.7 ± 1.7 b
	0.2	3.8 ± 0.3 ab	461.9 ± 38.7 a	44.3 ± 2.1 a	78.1	2.8 ± 0.3 ab	46.9	1.2 ± 0.2 a	6.5 ± 1.0 a
	0.5	**4.5 ± 0.4 b**	513.1 ± 44.1 a	41.4 ± 1.2 a	**84.4**	**3.4 ± 0.4 b**	34.4	0.5 ± 0.1 a	4.5 ± 0.9 a
'Belcanto'	0.05	3.4 ± 0.3 a	**463.6 ± 43.3 a**	45.3 ± 2.0 c	**75.0**	**2.3 ± 0.3 a**	**93.7**	**6.4 ± 0.5 d**	**9.1 ± 0.7 d**
	0.1	2.9 ± 0.4 a	449.8 ± 42.5 a	**46.9 ± 1.9 c**	65.6	2.1 ± 0.3 a	85.9	4.7 ± 0.5 c	7.0 ± 0.5 c
	0.2	3.1 ± 0.4 a	438.1 ± 53.3 a	34.4 ± 1.9 b	57.8	1.6 ± 0.3 a	25.0	0.8 ± 0.3 b	1.3 ± 0.3 b
	0.5	**3.4 ± 0.4 a**	443.9 ± 48.4 a	26.7 ± 1.6 a	65.6	2.2 ± 0.3 a	0	0 a	0 a
'Solaia'	0.05	1.7 ± 0.3 a	**422.8 ± 87.2 b**	**47.3 ± 3.1 b**	26.6	0.4 ± 0.1 a	**100**	**10.6 ± 0.4 c**	**14.1 ± 0.9 d**
	0.1	2.2 ± 0.3 a	355.3 ± 42.5 ab	44.2 ± 2.6 b	37.5	0.7 ± 0.1 ab	100	10.2 ± 0.5 c	11.1 ± 0.8 c
	0.2	1.7 ± 0.3 a	276.5 ± 39.8 ab	41.6 ± 2.4 b	**39.1**	**1.0 ± 0.2 b**	79.7	6.1 ± 0.8 b	5.5 ± 0.8 b
	0.5	**2.4 ± 0.3 a**	244.7 ± 28.8 a	28.9 ± 2.1 a	32.8	0.7 ± 0.2 ab	35.9	1.1 ± 0.3 a	2.2 ± 0.6 a

In this table abbrebviation SE denotes standard error

and 'Solaia' at 0.05 mg/l BA; 'Solaia' also having the highest number of roots per bulblet (10.6), while 'Aubade' had the longest roots (34.6 mm).

The highest percentage of bulblets forming new bulblets was 84.4% registered in 'Aubade' on 0.5 mg/l BAP, and this cultivar had the largest bulblets (4.5 mm diameter). The fresh weight of bulblets increased x6.7 in 'Aubade', x4.6 in 'Belcanto' and x4.2 in 'Solaia'.

In all cultivars, a decrease of all growth parameters with time was observed, including the success in new bulblet regeneration.

Histological analysis of SE and bulblet regeneration. To determine the early events and origin of regenerants in *Lilium* explants, histological studies were done by serial sectioning of material cultured at various TDZ concentrations. The dominant regeneration events were found to be connected with somatic embryogenesis. The regeneration process was not synchronized and after 5 weeks, SE at various developmental stages was found in the same material. Somatic embryos at the globular stage were found mostly in the surface explant layers. These early proembryogenic structures consisted of small isodiametric cells with dense dark-stained cytoplasm and large, conspicious nuclei (Fig. 6). In the same sections, more mature SE in the early heart-shape stage of development were also observed. Intensive cell divisions along the long axis of embryos led to more differentiation i.e pronounced polarity of embryos bringing them to the cotyledonary stage of development. At this stage emrbyos had a well-developed apical meristem, with leaf primordia and initial vascular elements visible. Later stages with typically elongated SE were absent. Instead we observed only small bulblets. It seems that SE originated from epidermal cells of initial explants and at the end of their formation there was no vascular connection with the initial leaflet explants. Somatic embryogenesis on TDZ-supplemented media was direct and we believe that SE here was of multicellular origin.

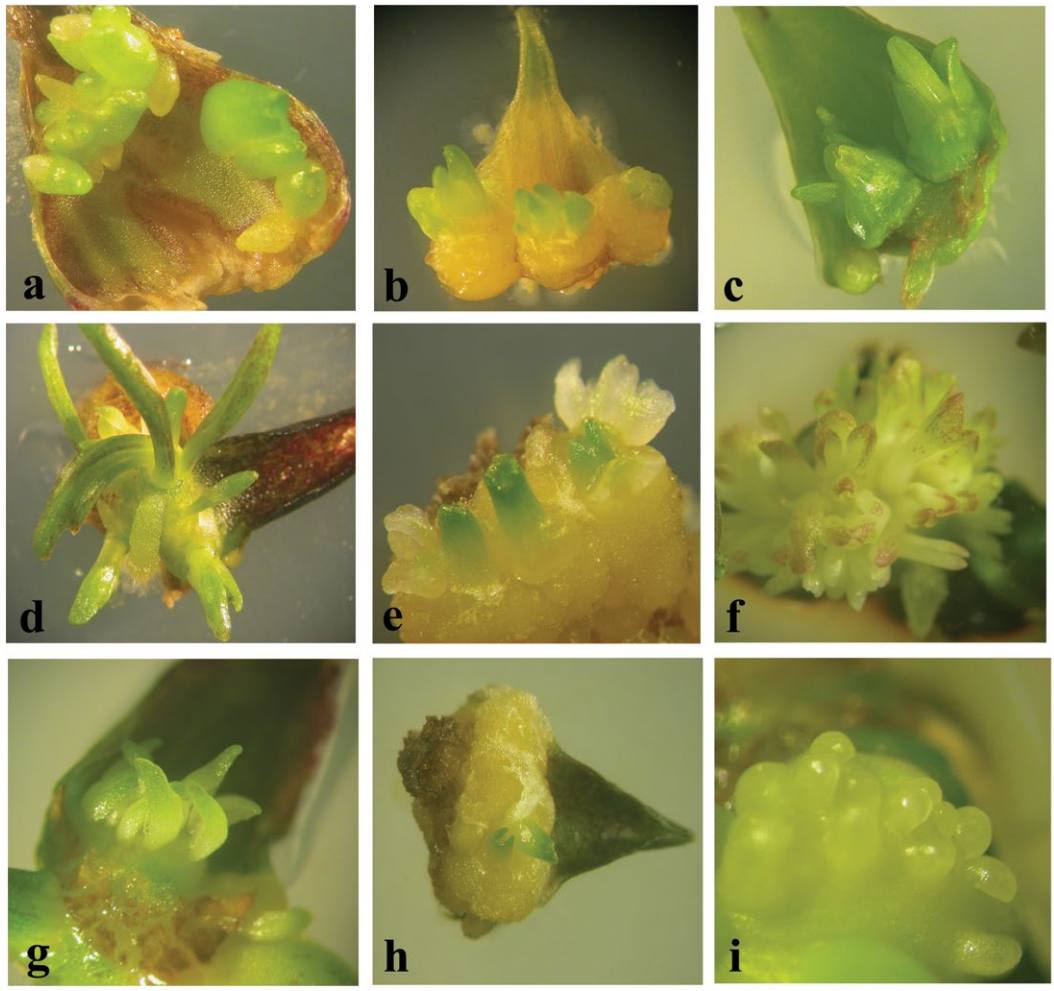

Fig 4. Induction and regeneration of somatic embryos and bulblets in oriental lily cultivars. Upper row cv. 'Aubade' a) SE developing on bulblet leaf explants cultured on 0.5 mg/l PIC, b) Large bulblet surrounded with a whorl of smaller bulblets, 3 mg/l PIC, c) Bulblets developing on the tip of bulblet leaf explant, 0.2 mg/l TDZ,
Middle row cv. 'Belcanto' d) cluster of interconnected bulblets on 0.5 mg/l PIC, e) bulblet regeneration on PIC 2 mg/l, f) bulblet clusters on bulblet leaf explants with 1 mg/l TDZ,
Lower row cv. 'Solaia', g) bulblets regenerating on basal portion of bulblet leaf explants, PIC 0.5 mg/l, h) proliferation on cut surface of bulb leaf tip PIC 2.0 mg/l, i) early stage regenerants on TDZ 0.5 mg/l.

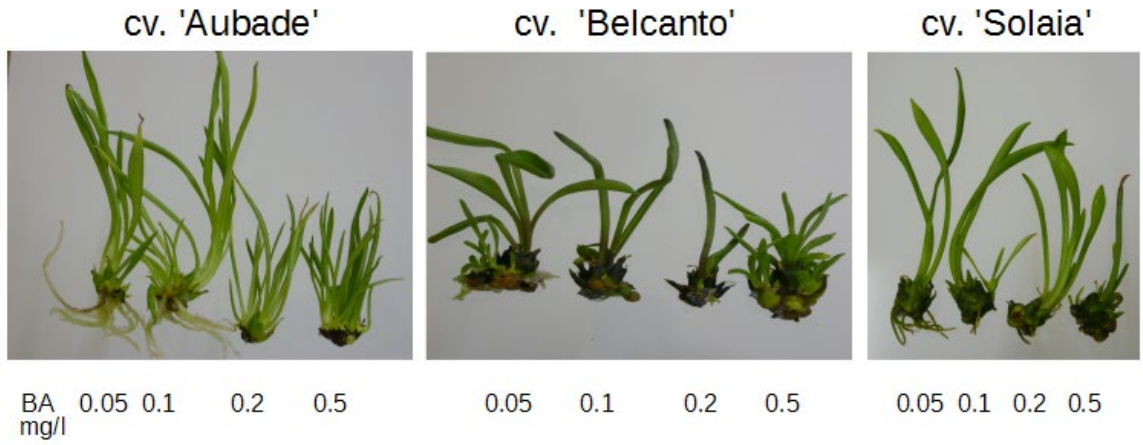

Fig 5. Further development of regenerated bulblets on media supplemented with 0.1 mg/l NAA and 0.05 – 0.5 mg/l BA.

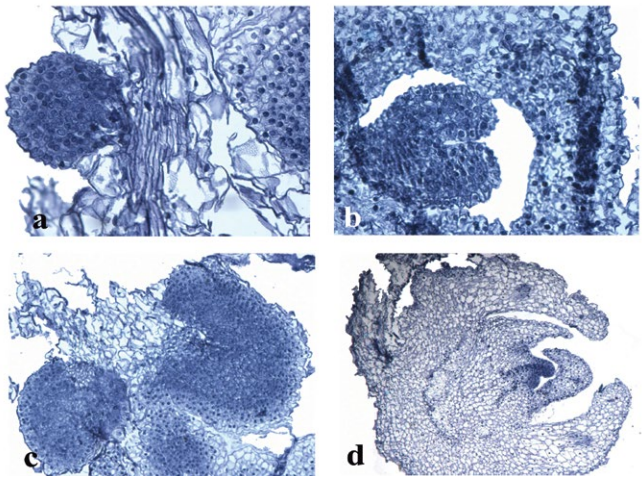

Fig 6. Histological analysis of somatic embryogenesis in bulb leaf explants of *Lilium sp.* a) Globular pro-embryo culture, b) and c) Early and late heart-shaped SE developmental, d) Longitudinal section of a cotyledonary stage (bulblet-like) embryo with well-delimited apical shoot bud and leaf primordial and initials of vascular elements.

DISCUSSION

Considering the concentration of plant growth regulators supplemented to the medium as a signal input and the regenerative response of plant explants as the response output, then it is obvious that in lilies the input signal is strongly buffered within the plant tissues. Thus, a regenerative process like somatic embryogenesis in *in vitro* bulblet scale explants occurred almost irrespectively of the type and concentration of applied plant growth regulators. From Figs. 1–3 it is evident that somatic embryogenesis needed no plant growth regulators for SE, as it occurred even on plant growth regulator-free medium. On the other hand, SE appeared both on media supplied with cytokinins (BA, TDZ) and auxin (picloram), which is contrary to the basic principle of hormonal regulation of differentiation in *in vitro*-cultured plant tissues dating back to the study of Skoog & Miller (1957), who postulated a balance of exogenous plant growth regulators as the main driving force of cell differentiation. Secondly, it is hard to imagine that three basic groups of plant growth regulators including cytokinins and auxins (here) and retardants (Kumar *et al.* 2005) may all exert the same kind of regeneration response within the same target tissue.

The nature of the factors or conditions leading cells to enter the SE pathway has been a subject of long, everlasting dispute to which we can contribute little from our present study. However, lilies are one of many groups of species which do not require plant growth regulators for SE. Nevertheless, there are also genotype differences, as cv. 'Belcanto' and 'Solaia' produced SE on plant growth regulator-free medium while cv. 'Aubade' did not. Similar genotype-related differences in the capacity of SE production were frequently observed in studies of SE in carrot (Krikorian 1982).

Plant growth regulators are not the only factors affecting SE in plants. Exogenous factors including light (Leshem *et al.* 1982; Pelkonen & Kauppi 1999), temperature (Van Aartrijk & Blom-Barnhoorn 1983) and internal factors such as mineral and carbohydrate nutrition also affect SE. However, the true merit of the *in vitro* culture technique is that factors/conditions can be kept constant, enabling only effects of plant growth regulators to be studied. In our study, the main goal was to investigate the contribution of cytokinins to the regeneration appearing in bulblet scale explants, comparing BA as the standard and TDZ as one of the most potent cytokinins. It is known that TDZ can induce both SE and shoot regeneration in the same explants (Huetteman & Preece 1993). We showed this to be true also for BA, as BA alone triggered callusing and SE on BA-supplemented media was therefore indirect.

Both cytokinin- and auxin-supplemented media were used for regeneration of lily bulblets in numerous older studies listed in the Introduction. Some of them even employed histological techniques, though surprisingly SE was not reported in those studies. Regeneration by SE and adventitious bud regeneration look similar by visual inspection, differences should be picked easily by histological studies. This opens the painful question of how reliable our knowledge is coming from older sources.

In the mid 1980s it was widely assumed that SE is induced by auxins while cytokinins have no vital role in this process (Pierik 1987). It is possible that investigators interested in practical applications and productivity did not pay much attention to the origin of regenerants, especially as they were not expected to develop on a cytokinin-supplemented medium. The failure of older studies to report SE can be perhaps attributed to genotypic differences. Even in the first study made by Haensch (1996), SE was observed only in a limited number of genotypes. Genome differences may be the reason why Khosravi *et al.* (2007) could not obtain SE in *Lilium longiflorum* on TDZ-supplemented medium and also why Han *et al.* (2005) and Xi *et al.* (2012) failed to observe SE in Orientals.

The problem seems to extend further back into the history of *Lilium in vitro* culture propagation. For instance, studies by Van Aartrijk and Blom-Barnhoorn (1981) and later describe adventitious bud formation in *Lilium speciosum* bulb scale explants but they don't mention SE formation on the same explants. Is it possible that these explants apart from large individual bulblets also contained typical SE as in our studies? Histological examination would find nothing suspicious, as SE production and adventitious bud regeneration are quite similar. However, the real reason for the failure to detect SE would then be the lack of late-stage elongated SE plantlets, as in *Lilium* SE there seems to be an early arrest of shoot elongation directly transforming early-stage somatic embryos into

small bulblets. Upon maturation, the SE origin of these bulblets/plantlets seems both unlikely and difficult to prove.

Our histological studies covered only TDZ-supplemented media and we should also test the effect of other growth regulators on early stages of SE. At the moment it seems at least in TDZ-induced SE that early SE structures, bulblets and plantlets belong to the same developmental line differing only in the time of their development. The lack of elongated late-stage SE and distribution of plantlet/early SE regenerants within Figs. 1-3 both support such a view.

Finally, this study shows that Oriental Lily cultivars follow a similar response pattern during *in vitro* culture and regeneration but there are also distinct differences in their regenerative potential.

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REFERENCES

Bacchetta L, Remotti PC, Bernardini C & Saccardo F. 2003. Adventitious shoot regeneration from leaf and stem nodes of *Lilium*. *Plant Cell, Tiss. Org. Cult.* **74:** 37-44.

Bakhshaie M, Babalar M, Mirmasoumi M & Khalighi A. 2010. Somatic embryogenesis and plant regeneration of *Lilium ledebourii* (Baker) Boiss., an endangered species. *Plant Cell, Tiss. Org. Cult*. **102:** 229-235.

El-Naggar H, Osman A & Sewedan E. 2012. *In vitro* propagation and organogenesis of *Lilium* 'Prato'. *African J. Biotech*. **11:** 14771-14776.

Gerrits M, Kim KS & De Klerk GJ. 1992. Hormonal control of dormancy in bulblets of *Lilium speciosum* cultured *in vitro*. *Acta Hortic*. **325**: 521-527.

Haensch K-T. 1996. Plant regeneration through somatic embryogenesis in different genotypes of *Lilium* hybrids. *Gartenbauwissenschaft* **62:** 214-218.

Han BH, Yae BW, Hee JY & Peak KY. 2005. Improvement of *in vitro* micropropagation of *Lilium* oriental hybrid 'Casablanca' by the formation of shoots with abnormally swollen basal plates. *Sci. Hortic*. **103:** 351–359.

Huetteman CA & Preece JE. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. *Plant Cell Tiss. Org. Cult.* **33:** 105–119.

Jensen WA. 1962. Botanical Histochemistry: Principles and Practice. WF Freemen and Company, San Francisco.

Jiménez V. 2005. Involvement of plant hormones and plant growth regulators on *in vitro* somatic embryogenesis. *Plant Growth Reg*. **47:** 91-110.

Kanchanapoom K, Ponpiboon T, Wirakiat W & Kanchanapoom K. 2011. Regeneration of lily (*Lilium longiflorum* 'Easter lily') by callus derived from leaf explants cultured *in vitro*. *Science Asia* **37:** 373-376.

Kapoor R, Kumar S & Kanwar JK. 2009. Bulblet production from node explants grown in *in vitro* in hybrid lilies. *Int. J. Plant Production* **3:** 1-6.

Khosravi S, Azghandi AV, Mojtahedi N & Haddad R. 2007. *In vitro* propagation of *Lilium longiflorum* var. Ceb-Dazzle through direct somatic embryogenesis. *Pak. J. Biol. Sci.* **10:** 2517-2521.

Kim SK, Lee JS, Haung KH & Ahn BJ. 2003. Utilisation of embryogenesis cell cultures for the mass production of bulblets in Oriental and Easter Lilies. *Acta Hortic*. **625:** 253-259.

Krikorian AD. 1982. Cloning higher plants from aseptically cultured cells and tissues. *Biological Reviews* **57:** 151-218.

Kumar S, Chaudhary V & Kanwar JK. 2008. *In vitro* propagation of oriental hybrid lily from root explants. *Adv. Hort. Sci*. **22:** 63-65.

Kumar S, Kashyap M & Sharma DR. 2005. *In vitro* regeneration and bulblet growth from lily bulbscale explants as affected by retardants, sucrose and irradiance. *Biol. Plant*. **49:** 629-632.

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*. *Sci. Hortic*. **17:** 129-136.

Liu L & Burger DW. 1986. *In vitro* propagation of Easter lily from pedicels. *HortScience* **21:** 1437-1438.

Murashige T & Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*. **15:** 473-497.

Nhut DT. 1998. Micropropagation of lily (*Lilium longiflorum*) via *in vitro* stem node and pseudo-bulblet culture. *Plant Cell Rep*. **17:** 913-916.

Nhut DT, Nguyen TD, Vu QL, Nguyen QT, Nguyen TM, Thai XD, Bui VL (2006). Standardization of *in-vitro* lily (*Lilium* spp.) plantlets for propagation and bulb formation. Proc. Intern. Workshop on Biotechnology in Agriculture. October 20-21: 134-138.

Niimi Y. 1985. Factors affecting the regeneration of growth of bulblets in bulbscale cultures on *Lilium rubellum* Baker. *J. Jap. Soc. Horti. Sci.* **4:** 82–86.

Novak FJ & Petru E. 1981. Tissue culture propagation of *Lilium* hybrids. *Sci. Hortic.* **14:** 191-199.

Parić A, Čakar J, Muratović E & Karalija E. 2011. Induction of bulblets on leaf and bulb explants of endangered *Lilium bosniacum* (G. Beck) G. Beck ex Fritsch. *Botanica Serbica* **35:** 31-35.

Pelkonen V-P & Kauppi A. 1999. The effect of light and auxins on the regeneration of lily (*Lilium regale* Wil.) cells by somatic embryogenesis and organogenesis. *Int. J. Plant Sci*. **160:** 483-490.

Pierik RLM. 1987. *In vitro* culture of higher plants. Martinus Nijhof Publishers, Dordrecht.

Robb MS. 1957. The culture of excised tissue of *Lilium speciosum* Thun. *J. Exp. Bot*. **8:** 348-352.

Sheridan WF. 1968. Tissue culture of monocot *Lilium*. *Planta* **82:** 189-192.

Simmonds JA & Cummings BG. 1976. Propagation of *Lilium* hybrids. II. Production of plantlets from bulb-scale callus cultures for increased propagation rates. *Sci. Hortic*. **5:** 161-170.

Skoog F & Miller CO. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symp. Soc. Exp. Biol*. **11:** 118–131.

Stimart DP & Ascher PD. 1978. Tissue culture of bulb-scale sections for asexual propagation of *Lilium longiflorum* Thunb. *J. Am. Soc. Hortic. Sci.* **103:** 182-184.

Tang YP, Liu XQ, Wahiti Gituru R &, Chen LQ. 2010. Callus induction and plant regeneration from *in vitro* cultured leaves, petioles and scales of *Lilium leucanthum* (Baker) Baker. *Biotechnol. & Biotechnol. Eq*. **24:** 2071-2076.

Takayama S & Misawa M. 1982. Regulation of organ formation by cytokinin and auxin in *Lilium* bulb scales grown *in vitro*. *Plant and Cell Physiol*. **23:** 67-74.

Takayama S & Misawa M. 1983. A scheme for mass propagation of *Lilium in vitro*. *Sci. Hortic*. **18:** 353-362.

Tribulato A, Remotti PC, Loffler HJM & Van Tuyl JM. 1997**.** Somatic embryogenesis and plant regeneration in *Lilium longiflorum* Thunb. *Plant Cell Rep*. **17:** 113–118.

Van Aartrijk J & Blom-Barhnoorn GJ. 1981**.** Growth regulator requirement for adventitious regeneration from *Lilium* bulb-scale tissue *in vitro*, in relation to duration of bulb storage and cultivar. *Sci. Hortic*. **14:** 261-268.

Van Aartrijk J. & Blom-Barnhoorn GJ. 1983. Adventitious bud formation from bulb-scale explants of *Lilium speciosum* Thunb. *in vitro.* Effects of wounding, TIBA and temperature. *Z. Pflanzenphysiol*. **110:** 355-363.

Van Aartrijk J, Blom-Barnhoorn GJ & Van der Linde PCG. 1990. Lilies. In Ammirato PV, Evans DA, Sharp WR & Bajaj YPS. (eds.), Handbook of Plant Cell Culture V5, pp. 535-576. Collier Macmillan Publishers, London.

Wickremesinhe ERM, Holocomb EJ. & Arteca RN. 1994. A practical method for the production of flowering Easter lilies from callus cultures. *Sci. Hortic*. **60:** 143-152.

Xi M, Fang L, Qiu S, Lu Y & Shi J. 2012, A high-efficiency regeneration system of oriental lily cultivar 'Constanta'. *Mol. Plant Breed*. **3:** 115-120.

REZIME

Različiti regenerativni potencijal kultivara orjentalnog ljiljana (*Lilium* sp.)

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Regenerativni potencijal *in vitro* razmnoženih lukovica istražen je kod tri komercijalne sorte Orijental ljiljana ('Aubade', 'Belcanto' and 'Solaia') u odnosu na dva sintetička citokinina BAP i TDZ kao i na pikloram regulator rastenja sa auksinskom aktivnošću. Listići sa *in vitro* lukovica su izolovani i kultivisani na MS podlogama sa 6-benzil aminopurinom (BAP 0-2.0 mg/l), thidiazuronom (TDZ 0-2.0 mg/l) ili pikloramom (PIC 0-3.0 mg/l). Kod sve tri sorte i u svim kombinacijama podloga eksplantati su nakon 5 nedelja regenerisali somatske embrione, lukovičice ili biljke. Dok je produkcija lukovičica bila izbalansirana produkcija biljaka i produkcija ranih somatskih embriona bile su komplementarne sa izrazitom produkcijom SE na višim koncentracijama regulatora rastenja i produkcijom biljaka na nižim koncentracijama. Pikloram je pokazivao jasnu regeneracionu demarkaciju sa niskom produkcijom biljaka na podlogama sa ili iznad 0.5 mg/l. BAP i TDZ su produkovali somatske embrione na svim koncentracijama regulatora rastenja uključujući i podloge bez regulatora rastenja. Podloge sa TDZ i BAP pokazivale su postepenu promenu od regeneracije lukovica na nižim prema SE na višim koncentracijama. Kod sva tri kultivara proces regeneracije praćen je histološki na podlogama sa TDZ i pokazano je prisustvo direktne SE. Stariji, izduženi stupnjevi SE su bili odsutni što ukazuje na ranu tranziciju somatskih embriona ljiljana u lukovičice. Optimalni uslovi za razmnožavanje razradjeni su i prikazani za sva tri sorte ljiljana.