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BUD CLUSTERS OF *Spathiphyllum* CULTIVARS: A NOVEL WAY TO PROPAGATE PEACE LILIES *in vitro*

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ABSTRACT

A special organ formation appearing on the inflorescences of *Spathiphyllum* cultivars, which has not been described so far in the literature was studied *in vivo* and *in vitro*. Histological examination revealed that they are not callus, but bud-like formulas or dwarf shoots growing in groups, which contain highly differentiated tissues, have a shoot axis, meristematic regions, and leaf primordia. These bud clusters have been shown to be propagable *in vitro* on both solid and liquid media, for which benzyladenine and thidiazuron cytokinins may be recommended. The process of shoot regeneration from these bud clusters was also investigated, which can be accelerated by inhibitors of gibberellin biosynthesis. This study presents a propagation method which is based on organogenesis, thus providing high genetic stability, while incorporating the advantages of liquid culture, allowing the further development of a simplified large-scale bioreactor propagation system for *Spathiphyllum* species.

Keywords: *Spathiphyllum*, anti-gibberellin, cytokinins, spadix inflorescence, liquid media culture, organogenesis

Abbreviations: BA – benzyladenine, BR – 6-benzylaminopurine riboside, FPR – flurprimidol, GA_3 – gibberellic acid 3, MS – Murashige & Skoog medium formula, MT – metatopoline, NAA – 1-naphthaleneacetic acid, PBZ – paclobutrazol, POD – peroxydase enzyme, TBZ – tebuconazole, TDZ – thidiazuron, UNZ – uniconazole

INTRODUCTION

Spathiphyllum species and hybrids are best known as ornamentals. In the past, some species of the genus were cultivated (e.g. *S. cannifolium*, *S. floribundum*), today we find mostly hybrid cultivars in trade. Their popularity is due to their ease of cultivation, the flowering of many varieties can be induced at any time with gibberellin treatment [Chen et al. 2002], and they have an excellent indoor tolerance, so they are widely used as an ornamental [Griffith 1998]. *Spathiphyllum* hybrids, in addition to flowering regularly even in low-light and low-humidity environments [Beytes and Hamrick 2003], are able to reduce the concentration of formaldehyde, benzene, and other volatile organic compounds found in indoor air [Liu et al. 2007, Soreanu et al. 2013]. While in 1980 there were only 6 varieties in cultivation, three decades later in 2010 more than 50 were available [Henny and Chen 2010]. The new varieties are produced by conventional breeding and selecting bud mutants from *in vitro* stocks [Chen et al. 2002]. According to commercial data [Van Den Berg 2010], the popularity of *Spathiphyllum* taxa is well indicated by the fact that for years they have reached the

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7th–10th rank in the market for houseplants in terms of turnover. Apart from small fluctuations, the volume traded has changed virtually little in each year over the past decade, as has the average price of the plant. Based on all this, these taxa are one of the most important representatives of the potted ornamental plant market. The first publications on the micropropagation possibilities of *Spathiphyllum* taxa appeared in the late 1970s [Kunisaki 1977, Fonnesbech and Fonnesbech 1979], and were one of the first ornamental plants to be produced by micropropagation [Griffith 1998]. Most published technologies use shoot culture to propagate *Spathiphyllum* taxa [Werbrouck et al. 1995, Dabski and Kozak 1997, Vargas and Garcia 1997, Watad et al. 1997, Chen 2000, Das et al. 2000, Han and Yae 2001, Ramirez-Malagon et al. 2001, Vardja and Vardja 2001, Wu 2001, García et al., 2015, Kacer et al. 2005, Özzambak et al. 2018, Fan et al. 2019], because indirect organogenesis through callus phase often leads to somaclonal variations in plants of the *Araceae* family [Chen and Henny 2006], however, some authors have also addressed somatic embryo cultures [Werbrouck et al. 2000, Zhao et al. 2012], since this method can produce much larger amounts of propagation material than shoot culture, required for plant regeneration after genetic transformation and is also much more suitable for bioreactor system cultivation than shoot cultures [Paek et al. 2001]. An efficient genetic transformation system based upon embryogenic suspension cell culture were also described in *Spathiphyllum* [Yu et al. 2016].

This study first presents the bud clusters appearing on the inflorescences of *Spathiphyllum*, the description of which has not been encountered in the literature so far, and then investigates their liquid culture propagation potential and shoot regeneration.

MATERIALS AND METHODS

Maintenance of mother plant stocks and establishment of *in vitro* cultures. Observation and analysis of bud clusters developing on the inflorescences of *Spathiphyllum* cultivars were carried out in the mother plant stock maintained for 4 years, and the inflorescences of these mother plants were used to establish *in vitro* cultures. Five cultivars of *Spathiphyllum*: 'Petite', 'Bellini', 'Cupido Compacto', 'Strauss' and 'Chopin' were studied. The plants were grown in a greenhouse where the minimum temperature was 22°C throughout the year. The number of cases in which a bud cluster appeared on the inflorescences were counted, and the number of all inflorescences were recorded. *In vitro* cultures were started from young spadices, still completely covered with spathes.

Histological examinations. For histological examinations, plant parts were fixed with Clarke fixative (75 v/v% absolute ethanol, 25 v/v% glacial acetic acid) and then dehydrated with Diapath Ottix Shaper, Diapath Ottix Plus solutions and infiltrated with ParaMat Extra Gurr (solidification temp: 58°C) according to the protocol issued for Ottix solutions. From the samples cast into paraffin blocks, 12 µm thick slices were prepared with a Thermo Scientific Microm HM355 rotary microtome and stained by the Sass method (Basic red 2 and Fast green FCF) or the Sharman method (Basic red 2 and Orange G) [Ruzin 1999], sections were examined and imaged with a Euromex iScope IS.1153-PLi light microscope.

***In vitro* propagation.** *Spathiphyllum* 'Petite' bud clusters were propagated after their *in vitro* appearance on agar-solidified medium (0.5 mg l^{-1} BA and 0.1 mg l^{-1} NAA on half strength MS medium). To investigate the multiplication on liquid medium the effect of different cytokinins (BA, BR, MT, TDZ) were examined at various concentrations (0.05, 0.1, 0.2, 0.5 mg l^{-1}), in each case with 0.1 mg l^{-1} NAA supplementation, the base of the medium was non-solidified half strength MS formula supplemented with 20 g l^{-1} sucrose, the pH was adjusted to 5.7. Light for the *in vitro* cultures were provided by fluorescent illumination with an intensity of 3000 lux, and a 16-hour daylength, the temperature was at 25°C on average. Three buds isolated from the clusters were placed in each 100 ml (20 cm long, 3 cm diameter) glass tubes, sealed with 3 layers of cling film, in each treatment 16 tubes were used and rotated by a tissue culture rotator (custom made, Óbuda TSZ, HU) at a speed of 20 rpm, the rotor drum angle was fixed at 30 degrees. The plants were evaluated after 2 months, the number of buds per cluster was counted and the status of the cluster was determined by categorization. To determine the status of the clusters they were classified to the following classes: 1. dead clusters, 2. clusters with at least half browning, 3. healthy, developing buds with green leaf primordia, with a white part at the apex, 4. clusters showing signs

Table 1. Effect of different cytokinins in liquid media on the bud clusters

Cytokinin (mg l^{-1})	Bud number[1] (pcs)			Peroxydase activity[1] (U g^{-1})		State category (%)[2]						
						1	2	3	4	5	Homogenous group	
0.05 BA	4.6	cde	ab	12.9	a	0.0	0.0	96.4	3.6	0.0	ab	a
0.1 BA	6.3	abcd				0.0	0.0	82.6	17.4	0.0	ab	
0.2 BA	9.3	a				0.0	5.7	68.6	17.1	8.6	a	
0.5 BA	8.8	ab				0.0	58.8	41.2	0.0	0.0	d	
0.05 BR	3.7	ef	b	30.4	b	0.0	13.3	83.3	3.3	0.0	abc	b
0.1 BR	4.4	def				3.6	0.0	89.3	7.1	0.0	ab	
0.2 BR	3.7	ef				0.0	12.2	80.5	4.9	2.4	abcd	
0.5 BR	3.5	cdef				17.6	23.5	58.8	0.0	0.0	cd	
0.05 MT	4.5	cdef	ab	63.2	c	17.9	0.0	82.1	0.0	0.0	bcd	ab
0.1 MT	4.4	cdef				0.0	6.7	93.3	0.0	0.0	abc	
0.2 MT	5.8	bcd				14.3	17.1	57.1	11.4	0.0	abcd	
0.5 MT	3.1	f				0.0	6.3	87.5	6.3	0.0	abc	
0.05 TDZ	6.2	abcdef	a	14.6	a	0.0	23.1	53.8	15.4	7.7	abc	ab
0.1 TDZ	7.4	abcdef				0.0	0.0	92.6	7.4	0.0	ab	
0.2 TDZ	5.1	bcdef				7.7	34.6	53.8	3.8	0.0	cd	
0.5 TDZ	7.2	abc				0.0	6.7	80.0	10.0	3.3	ab	

[1]Different letters denote significant differences between treatment groups. Games-Howell multiple comparison test, $p \leq 0.05$
[2]Different letters denote significant differences between treatment groups. Dunn-Bonferroni multiple comparison test, $p \leq 0.05$

of the onset of shoot regeneration (green leaf primordia are looser, they are larger in size, cover the apex of the bud, or are already beginning to expand, and between them whitish leaf blades extend upwards), 5. clusters containing parts regenerated into complete shoots. The effect of stress on bud clusters during liquid culturing was investigated by measuring peroxidase levels spectrophotometrically by Shannon et al. [1966] method.

Investigation of shoot regeneration from bud clusters. The study of shoot regeneration from buds was first started on half strength MS medium solidified with 5.5 g l^{-1} plant agar (Duchefa BV), supplemented with 0.25 mg l^{-1} BA together with 6 different levels of NAA in the range of 0–1.5 mg l^{-1}, then the effect of NAA in 4 different levels (0.25–2 mg l^{-1}) was tested together with 3 mg l^{-1} metatopoline and 3 mg l^{-1} GA_3 (Tab. 2, treatments 1–10). The status of the bud clusters was evaluated after 6 months (after 3 months the medium was changed, the clusters were not divided) according to the category system described in the previous section. In addition, the effect of elevated macronutrient levels (1 and 2 × MS macronutrient concentrations) and sucrose (20 and 40 g l^{-1}, respectively) was examined after 2 months (Tab. 2, treatments 11–15). The effect of giberrellin biosynthesis inhibitors was also tested with 4 different compounds: paclobutrazol, uniconazole, tebuconazole and flurprimidol. Paclobutrazol was tested in the range of 0–2 mg l^{-1} without growth regulators, with 0.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA and 1 mg l^{-1} BA + 0.1 mg l^{-1} NAA. Uniconazole, tebuconazole and flurprimidol were tested together with other growth regulators in the concentration range of 0–2 mg l^{-1}, details can be seen in Table 3, treatments 16–45.

Statistical evaluation. For statistical evaluation of the measured data, robust Welch analysis of variance test was used, with Games-Howell pairwise comparison tests at 95% significance level. Data categories generated by classification (bud cluster states) were analyzed using the nonparametric Kruskal-Wallis H test and Dunn-Bonferroni pairwise comparisons were performed to point out the differences in the state category distribution of the clusters.

Table 2. Effect of different PGRs on the shoot regeneration of the bud clusters

Treatment	Medium base (MS)	Sucrose (g l^{-1})	Cytokinin mg (l^{-1})	NAA (mg l^{-1})	GA_3 (mg l^{-1})	Months	State category (%)					Homogenous groups[1]
							1	2	3	4	5	
1	0.5×	20	0.25 BA	0	–	6	0.0	0.0	76.2	7.1	16.7	a
2	0.5×	20	0.25 BA	0.5	–	6	12.9	6.5	45.2	12.9	22.6	ab
3	0.5×	20	0.25 BA	0.75	–	6	2.8	8.3	83.3	0.0	5.6	abc
4	0.5×	20	0.25 BA	1	–	6	11.4	11.4	48.6	20.0	8.6	bc
5	0.5×	20	0.25 BA	1.25	–	6	12.0	36.0	40.0	12.0	0.0	abc
6	0.5×	20	0.25 BA	1.5	–	6	40.0	20.0	26.7	13.3	0.0	c
7	0.5×	20	3 MT	0.25	3	6	2.8	0.0	86.1	11.1	0.0	a
8	0.5×	20	3 MT	0.5	3	6	0.0	2.8	88.9	5.6	2.8	a
9	0.5×	20	3 MT	1	3	6	0.0	10.0	80.0	6.7	3.3	a
10	0.5×	20	3 MT	2	3	6	0.0	14.0	69.8	9.3	7.0	a
11	0.5×	–	0.5 BA	0.1	–	2	2.0	40.8	42.9	14.3	0.0	a
12	1×	20	0.5 BA	0.1	–	2	0.0	0.0	85.7	14.3	0.0	b
13	2×	20	0.5 BA	0.1	–	2	0.0	0.0	95.5	4.5	0.0	ab
14	1×	40	0.5 BA	0.1	–	2	0.0	3.0	87.9	6.1	3.0	b
15	2×	40	0.5 BA	0.1	–	2	0.0	0.0	96.9	3.1	0.0	b

1Different letters denote significant differences between treatment groups. Dunn-Bonferroni multiple comparison test, $p \leq 0.05$
MS – Murashige Skoog medium formula, BA – benzyladenine, NAA – naphthalene acetic acid, GA3 – gibberellic acid 3, MT – meta-topoline

RESULTS AND DISCUSSION

Description and characterization of bud clusters. According to previous observations when using spadices of *Spathiphyllum* cultivars to start a sterile culture *in vitro*, they sometimes form on their surface small but visible spherical tissue formations with an average diameter of 3–5 mm instead of normal shoots (Fig. 2, middle row). These spherical formulas are dwarf-stemmed shoots covered with green on the outside and whitish-colored leaf primordia on the inside, according to histological examinations they have a meristematic region (middle right in Fig. 1), resembling a bud. In addition to their *in vitro* occurrence, the development of these formations was also observed *in vivo* on spent, greening spadices. Histological examination revealed that the stem axis of the *in vivo* developed clusters is directly connected to the inflorescence stem axis. The leaf primordia and stem axis of the formula already contain differentiated tissues, vascular elements can be observed in the stem part (Fig. 1, left). The leaf primordia have a thickened-walled single-row epidermis, stomatal pores are also found on the epidermis, and excretory idioblasts can be observed sporadically in the mesophyllum of the leaf primordia, with calcium oxalate raphide crystals characteristic to aroid species (Fig. 1, lower right). There is an apical meristem at the apex of the stem axis, and axillary bud meristems develop at the base of the leaf primordia at the top of the stem (Fig. 1 left). In a more advanced state, the formulas can be detached from the spadices and can be further grown *in vitro*, they can grow on both solid and liquid media, and the buds form larger clusters (bottom row of Figure 2). Shoot regeneration can also be observed from the buds *in vitro*, during which the formulas develop into normal sized *in vitro* shoots (Fig. 3). No further division or transformation into shoot was observed *in vivo* on the mother plants if the clusters were left on the spadices. During the decline of the spadix the clusters were also killed, but if detached from it and planted in peat medium, they survived for another one month, but no growth and shoot development were observed. However, in an *in vitro* environment, they can be propagated well on media supplemented with carbohydrates and growth regulators. Axillary bud meristems differentiate at the base of the leaf primordia found in the top of the bud formula and new buds are formed. The buds are initially

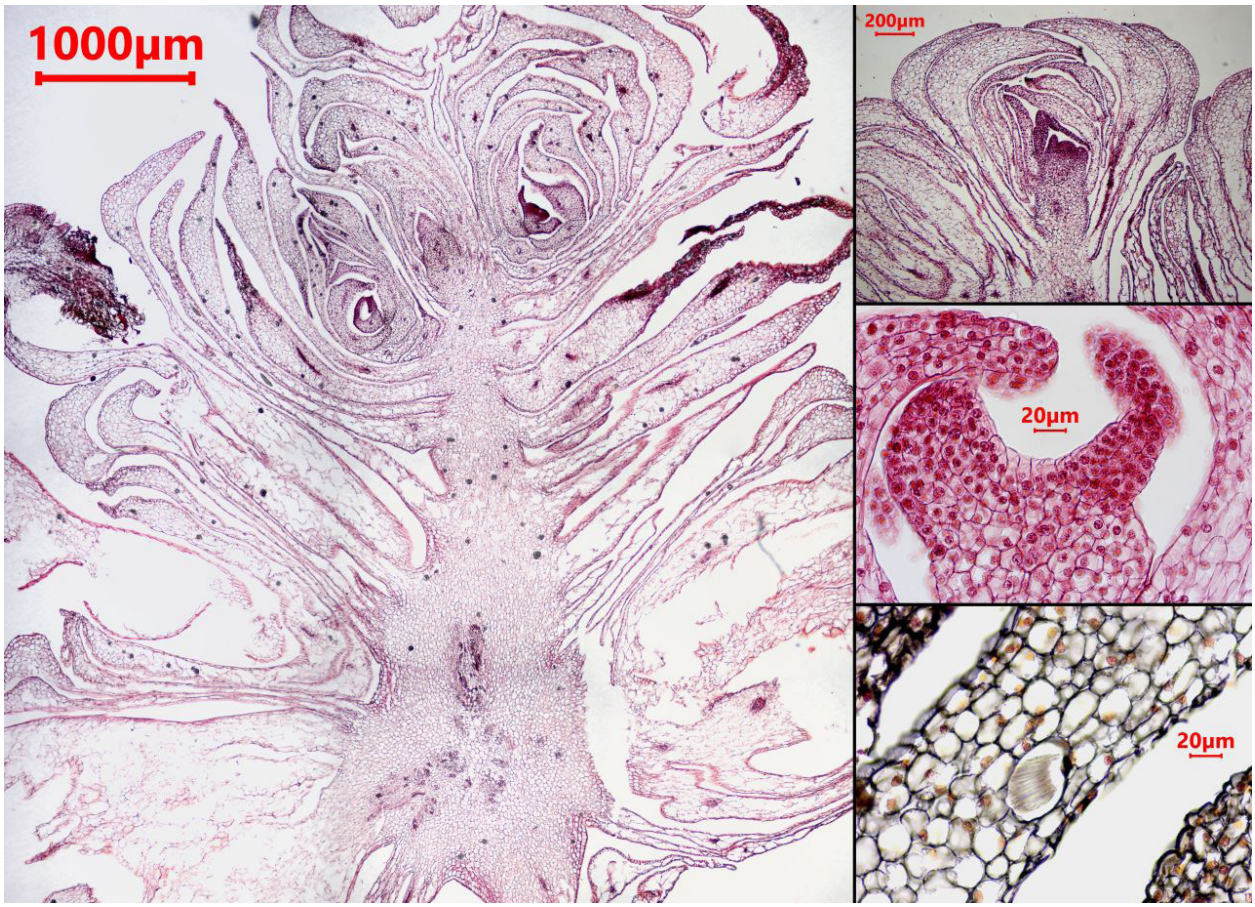

Fig. 1. Cross – sectional images of bud clusters. Left: *in vivo* developed bud cluster on the spadix of 'Strauss' cv. (4×, SASS staining, 12 µm). Upper right: *in vitro* developed bud cluster on 'Petite' cultivar (10×, SASS staining, 12 µm). Right middle: meristem of *in vitro* developed cluster, 'Petite' cultivar (40×, SASS staining, 12 µm). Lower right: 'Strauss' cultivar, leaf primordium of *in vivo* cluster (40×, SHARMAN staining, 12 µm)

closely spaced to form a more or less spherical colony (Fig. 2, bottom row), and then, as more and more buds and branches develop, the colony loosens and disintegrates during passage or even spontaneously into smaller units. The not yet disintegrating clusters can also be cut to one bud segments, the spherical shoot tips and buds continue to grow on their own, placed one by one on the medium.

Frequency of occurence of the bud cluster formula. Out of the five cultivars examined the development of the formula *in vivo* or *in vitro* could be observed on four. Considering the data of 4 years, compared to the total number of inflorescences, the proportion of inflorescences which showed bud cluster in the case of 'Petite' cultivar varied from 0 to 5.8% (n = 39–104), in the case of 'Strauss' cultivars 0–4.7% (n = 32–64). On 'Bellini' and 'Cupido Compacto' cultivars the formation could be observed only in *in vitro* environment. When morphologically normally developed spadices were placed on *in vitro* medium, the formula appeared 8 times *in vitro*, out of the 217 spadices, this is 3.7% of all cases. Inducing media included both cytokinin-predominant and auxin-predominant media. In half of the cases, bud cluster formation was observed with 3 mg l^{-1} MT and 0.1 mg l^{-1} NAA ('Petite'), but also observed in one case with 8 mg l^{-1} MT + 0.1 mg l^{-1} NAA ('Cupido Compacto'), in one case at 0.75 mg l^{-1} BA + 0.05 mg l^{-1} NAA ('Bellini'), and also at 4 mg l^{-1} 2,4-D + 1 mg l^{-1} BA and 0.1 mg l^{-1} 2,4-D + 1 mg l^{-1} BA ('Petite'). The bases of

the media in each case were half strength MS supplemented with 20 g l^{-1} sucrose. The appearance of bud clusters, even with very different hormone ratios, suggests that they do not develop primarily under the influence of growth regulators, but may already be present in the spadix placed on the medium, and appear in the *in vitro* environment due to the further development of the detached spadix. Bud clusters appearing on *in vivo* plants, even in the absence of growth-regulating treatments, also support this.

In vitro propagation on liquid medium

The results of the liquid culture experiment examining the effect of different cytokinines are shown in

Fig. 2. *In vivo* and *in vitro* bud clusters of *Spathiphyllum* cultivars. Top row right: an adult bud cluster on spent and green spadix of the 'Petite' cv. Top row left: an *in vivo* developing bud on the green spadix of the 'Petite' cv., photographed under a stereomicroscope. Middle row: *in vitro* developed bud clusters on spadices placed on medium of 'Petite', 'Strauss' and 'Bellini' cvs. Bottom row: bud clusters of the 'Petite' cv. grown *in vitro*, left: on solid medium, middle: on liquid medium, right: bud cluster showing signs of shoot regeneration and rooting on solid medium

Fig. 3. Process of shoot regeneration from buds detached from bud clusters of 'Petite' cultivar and peroxidase enzyme activity in each phase (Games-Howell test, $p \leq 0.05$)

Table 1. The primary indicator of multiplication in this formula is the increase of bud number within the clusters. The highest multiplication rate could be observed using the two higher concentrations of BA and at every level of TDZ. In the case of TDZ no significant difference can be detected between the concentrations. Analyzing the peroxidase activity measured from the buds of state category 3, it can be concluded that there is a significant difference between the groups according to the cytokinin types. The lowest POD activity was measured in the BA and TDZ groups, where the rate of proliferation was the highest, and a significantly higher stress effect was measured in the BR and especially in the MT group. Examining the distribution of bud cluster status, there are treatments in the BA and TDZ groups that result in zero or minimal bud death. There is a significant difference between the mean status distributions of the treatments grouped according to cytokinines: BA causes minimal bud death in the stock, there is a high proportion of category 3 or 4 clusters (multiplicating buds or clusters showing signs of shoot regeneration), while BR shifts the distribution to the other direction: the proportion of buds that died or began to perish is larger. The MT and TDZ groups are in a transient state between the two endpoints, with no significant differences from either. The use of 0.2 mg l^{-1} BA or 0.1 mg l^{-1} TDZ is recommended for propagation.

In the shoot cultures of *Spathiphyllum* most author found BA to be ideal of the tested cytokinins for propagation on solid medium at a concentration of 1–2 mg l^{-1} [Vargas and Garcia 1997, Chen 2000, Das et al. 2000, Han et Yae 2001, Ramirez-Malagon et al. 2001, Vardja and Vardja 2001, Wu 2001, Kacer et al. 2005, Dewir et al. 2006], however Werbrouck et al. [1995] point out that BA accumulates in the basal part of *Spathiphyllum* shoots and can later cause problems during rooting. Fewer growth regulators are sufficient for the same effect in a liquid medium compared to a solid one because the diffusion of solutes in liquid environment is better compared to an agar-solidified medium [Ascough and Fennell 2004]. The results of

Dewir et al. [2006] support this also in *Spathiphyllum* shoot cultures.

The multiplication rate for the two better culturing method is 9.4-fold for 8 weeks in both cases, and buds weighing an average of 131 mg can be obtained. The multiplication rate of *Spathiphyllum* 'Petite' in *in vitro* shoot culture on solid medium by other authors: 5.8 pcs/6 weeks with 116 mg/shoot and 11.6 pcs/6 weeks with 39 mg/shoot [Ramirez-Malagon et al. 2001], 10.1 pcs/40 days with 38 mg/shoot [Watad et al. 1997], in liquid medium Watad et al. [1997] also reached a rate of 14.2 pcs/25 days, but with an average weight of only 38 mg/shoot. Direct comparison of multiplication rates is difficult due to different durations and specific weights, however it can be said that the rate obtained with the presented method fits the literature data. This method requires less work compared to shoot cultures and does not require special equipment used for liquid cultivation of *Spathiphyllum* shoot cultures [Watad et al. 1997, Dewir et al. 2006]. In addition, it results in propagules with high specific weight, so they can be more easily develop further or more easily acclimatized due to the more stored nutrients. The method recommended by Dewir et al. [2006] combines large-scale bioreactor culture with shoot culture in which shoot cultures are grown in modified air-lift reactors in liquid medium, and Kacer et al. [2005] also show a similar solution with shoot cultures, where the risks of somaclonal variability are reduced because of using the differentiated plant organs. However growing shoot cultures on liquid medium often leads to hyperhydration problems in case of continuous immersion [Ascough and Fennell 2004], thus, to avoid this, in practice the shoots are lifted from the liquid medium by some technical solution (application of spacers or impregnation of a porous surface with nutrient solution) to ensure their aeration or temporary immersion can be used [George et al. 1993]. In the case of *Spathiphyllum* bud clusters, this is not necessary because they are able to float on the surface of the liquid, and due to the spherical shape of the clusters, they also withstand the mechanical stress effects caused by medium agitation in liquid culture systems.

Shoot regeneration from the bud clusters

Leafy shoots with normal morphology and the same appearance as *in vitro* shoot cultures can develop from the bud-like formulas, which, like *Spathiphyllum* shoot cultures, can be easily acclimatized. *In vitro* shoots regenerated from bud cluster cultures of 'Petite' and 'Cupido Compacto' cultivars were successfully acclimatized and grown to mature, flowering plants. According to the observations on the onset of shoot regeneration, the frequent division of clusters is not conducive to the process, and the duration of regeneration is prolonged in some cases (1–3 months). Factors playing part in the process are to be the subject of further investigations. The shoot regeneration process can be divided into 6 phases, which are as follows:

1. Pre-regeneration condition, the bud is spherical, the top is white, lateral leaf primordia are green;
2. The lateral green leaf primordia are overgrowing the top, covering the upper white part like cabbage leaves;
3. The apex opens, greenish-white developing leaves emerge from the white part;
4. The greenish-white developing leaves begin to turn green;
5. The young leaves begin to protrude from the center of the bud, the leaf lamina is unrolling;
6. The shape of the leaf lamina becomes visible, but the petiole is still short;
7. The petiole is elongated and the shoot stem becomes also visible.

Root formation always occurs in phase 6, but it could be observed in some cases as early in phase 2. Measured in each phase, peroxidase activity increases as shoot development progresses and then decreases upon completion (Fig. 3).

When examining the effect of different NAA concentrations at low cytokinin level (0.25 mg l^{-1} BA), shoot regeneration was observed after 6 months on solid medium (Tab. 2, treatments 1–6), to the greatest extent on media with 0 mg l^{-1} NAA. As the NAA concentration increased, the rate of shoot regeneration deteriorated, with a higher rate of cluster deaths. However, at high cytokinin (3 mg l^{-1} MT) and gibberellin (3 mg l^{-1} GA_3) levels, no difference was observed between the different NAA concentrations, neither in terms of cluster death nor shoot regeneration, the bud clusters actively proliferated and did not regenerate into shoots (Table 2, treatments 7–10). Lack of saccharose also results in significant cluster deterioration as early as 2 months, but neither excess sucrose nor excess macronutrient has a significant effect on the

change in cluster status after this time (Tab. 2, treatments 11–15).

Because GA_3 was more likely to inhibit shoot conversion than auxin (Tab. 2, treatments 7–10), therefore, we tested several triazole-type compounds that cause, among other things, inhibition of gibberellin biosynthesis in plants, to examine whether they promote shoot regeneration on solid media (Tab. 3, treatments 16–45). Based on the results, some of these gibberellin biosynthesis inhibitors caused shoot regeneration already after 3 months. In the absence of cytokinin and auxin, PBZ induced more significant regeneration at low concentrations (0.25 mg l^{-1}) (phases 4 and 5 combined: 28.2%) as early as 3 months, but as the concentration increased, an increasing proportion of bud clusters died (Tab. 3, treatments 16–19). Addition of 0.5 mg l^{-1} BA to PBZ treatments decreases the mortality rate with increasing PBZ concentration (Tab. 3, treatments 20–23), and with 1 mg l^{-1} BA addition, this effect disappears (Tab. 3, treatments

Table 3. Effect of different GA inhibitors on the shoot regeneration of the bud clusters

Treatment	BA (mg l^{-1})	NAA (mg l^{-1})	GA inhibitor (mg l^{-1})	State category (%)					Homogenous groups[1]	
				1	2	3	4	5		
16	–	–	0.25 PBZ	6.5	2.2	63.0	13.0	15.2	a	d
17	–	–	0.5 PBZ	20.0	35.6	35.6	4.4	4.4	b	
18	–	–	1 PBZ	35.4	52.1	4.2	8.3	0.0	b	
19	–	–	2 PBZ	97.9	2.1	0.0	0.0	0.0	c	
20	0.5	0.1	0.25 PBZ	0.0	0.0	59.1	22.7	18.2	a	ab
21	0.5	0.1	0.5 PBZ	4.5	0.0	75.0	18.2	2.3	ab	
22	0.5	0.1	1 PBZ	8.9	16.1	50.0	23.2	1.8	b	
23	0.5	0.1	2 PBZ	16.3	44.2	32.6	7.0	0.0	c	
24	1	0.1	0.0313 PBZ	5.6	0.0	86.1	8.3	0.0	ab	ab
25	1	0.1	0.0625 PBZ	0.0	0.0	90.9	9.1	0.0	ab	
26	1	0.1	0.125 PBZ	0.0	16.7	63.9	19.4	0.0	ab	
27	1	0.1	0.25 PBZ	1.2	0.0	75.9	12.0	10.8	a	
28	1	0.1	0.5 PBZ	0.0	14.9	73.1	10.4	1.5	b	
29	1	0.1	1 PBZ	0.0	20.6	77.9	0.0	1.5	b	
30	1	0.1	2 PBZ	0.0	23.5	73.5	2.9	0.0	b	
31	1	0.1	0.0313 UNZ	0.0	0.0	75.0	25.0	0.0	a	b
32	1	0.1	0.0625 UNZ	0.0	0.0	91.7	8.3	0.0	a	
33	1	0.1	0.125 UNZ	0.0	0.0	86.1	13.9	0.0	a	
34	1	0.1	0.25 UNZ	6.9	1.4	86.1	4.2	1.4	ab	
35	1	0.1	0.5 UNZ	5.6	8.3	77.8	8.3	0.0	ab	
36	1	0.1	1 UNZ	15.2	18.2	63.6	3.0	0.0	bc	
37	1	0.1	2 UNZ	33.3	18.5	48.1	0.0	0.0	c	
38	1	0.1	0.0313 TBZ	0.0	5.6	69.4	13.9	11.1	a	a
39	1	0.1	0.0625 TBZ	2.8	8.3	72.2	8.3	8.3	a	
40	1	0.1	0.125 TBZ	0.0	5.6	66.7	16.7	11.1	a	
41	1	0.1	0.25 TBZ	0.0	2.8	77.8	8.3	11.1	a	
42	0.2	0.1	0.05 FPR	22.2	11.1	48.1	18.5	0.0	ab	c
43	0.2	0.1	0.1 FPR	6.7	16.7	50.0	26.7	0.0	a	
44	0.2	0.1	0.2 FPR	13.3	20.0	50.0	16.7	0.0	a	
45	0.2	0.1	0.5 FPR	22.2	55.6	18.5	3.7	0.0	b	

[1]Different letters denote significant differences between treatment groups. Dunn-Bonferroni multiple comparison test, $p \leq 0.05$
BA – benzyladenine, NAA – naphthalene acetic acid, GA – gibberellin, PBZ – paclobutrazol, UNZ – uniconazole, TBZ – tebuconazole, FPR – flurprimidol

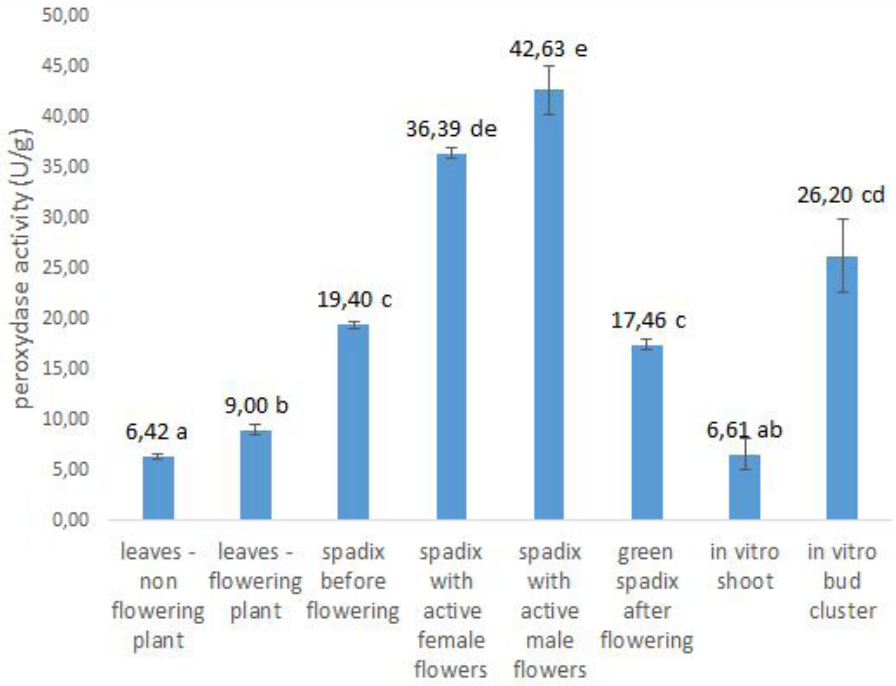

Fig. 4. Peroxydase activity in leaves and in inflorescence of Petite cultivar in different stages. Different letters denote significant differences (Games-Howell test, $p \leq 0.05$)

24–30), and shoot regeneration is highest in both cases at 0.25 mg l^{-1} PBZ. In the study of uniconazole, lower concentrations initiated shoot regeneration (phase 4 clusters), at the lowest concentration of 0.03125 mg l^{-1} the proportion of phase 4 clusters was 25%, but after 3 months there was no fully regenerated shoot, however, with the increase of concentration mortality of bud clusters also increased here (Tab. 3, treatments 31–37). There was no significant difference between tebuconazole treatments – there was a good rate of regeneration at all concentrations (16.7–27.8%) and minimal mortality (Tab. 3, treatments 38–41). Flurprimidol was tested at lower cytokinin levels than the other treatments, and the results showed that the mortality rate was also high here just like in the PBZ treatments at low cytokinin levels. We have no data at higher cytokinin level, so it is not clear whether the decay is due to lower cytokinin level or flurprimidol itself (Tab. 3, treatments 42–45). Regarding the treatment groups, the best shoot regeneration with the lowest mortality out of the 4 gibberellin biosynthesis inhibitors was reached with the treatments 1 mg l^{-1} BA + tebuconazole (Tab. 3, treatments 38–41), or 0.5 and 1 mg l^{-1} BA + paclobutrazole should be highlighted (Tab. 3, treatments 20 and 27), where a concentration of paclobutrazole is optimal at 0.25 mg l^{-1}. It can be established that triazoles result in faster and greater shoot regeneration from buds.

The peroxidase levels of *in vitro* bud clusters showing no signs of shoot regeneration were always higher than those measured in leaf *in vivo* or in the leaves of *in vitro* shoot cultures, they are statistically equal to those measured in the spadix before or after flowering (Fig. 4). On the other hand, the peroxidase level of buds starting shoot regeneration decreases to the same range which can be measured in leaves *in vivo*, or *in vitro* shoots (Fig. 3). The high peroxidase activity of bud clusters may be explained by the formation of these formulas on the spadix, so the oxidative environment in them may be similar. During the generative phase of the plants the proportions and levels of growth regulators are altered and in *Spathiphyllum* this causes a higher oxidative environment as described by Dewir at al. [2007]. They found that gibberellic acid-treated plants have higher peroxidase activity, and an increase in glutathione levels under oxidative stress triggers

flowering, therefore they consider the increase in GA levels essential for switching between the vegetative and generative phases. GA contributes to flowering induction in many cases, but not in all species [Barbosa and Dornelas 2020], however, the flowering inducing effect of GA_3 has been described in several cases in plants belonging to the *Araceae* family [Henny et al. 1999, Chen et al. 2003, Subbaraj et al. 2010]. Werbrouck et al. [1996] have shown that imidazole and triazole-type fungicides not only inhibit gibberellin synthesis in *Spathiphyllum* but also can significantly alter the metabolism of cytokinins. The current results suggest that the bud clusters developed originally on the spadix have a growth regulatory environment characteristic to the generative phase, and the gibberellin biosynthesis inhibitors promote changes effectively in this regulatory environment making it similar to a vegetativ phase by regulating endogenous gibberellin synthesis and this accelerate the process of shoot regeneration from the clusters.

CONCLUSIONS

Micropropagation processes on solid media with shoot culture require quite a lot of manual labor, so they are expensive. Liquid culture can reduce costs, because it makes the process partially or completely automated, in addition to having a positive effect on plant development [Paek et al. 2001, Grzegorczyk-Karolak et al. 2021]. Liquid culture propagation experiments open up the possibility of using bioreactors, which are now increasingly used for *in vitro* propagation of plants as well [Paek et al. 2005]. According to the current results, the bud clusters found in *Spathiphyllum* cultivars are excellent subjects for liquid culturing, they do not require the use of spacers or any other technical addition during liquid culture because they are able to float on the surface of the liquid medium. The propagation method presented here operates using an organogenetic developmental pathway, thus minimizing the risk of somaclonal variability. The bud clusters can be used in a two-step propagation process where the propagation phase takes place in a liquid medium in a bioreactor and the shoot regeneration is carried out on a solid medium similar to the methods used for orchid propagation with protocorm or protocorm like bodies [Chugh et al. 2009]. Due to their size and shape, they may also be suitable for use as an artificial-somatic seed after encapsulation, in which case they may be used immediately after bioreactor propagation without the need of acclimation when encapsulated with a suitable growth regulator combination for shoot regeneration.

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