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The plant activator BTH promotes *Ornithogalum dubium* and *O. thyrsoides* differentiation and regeneration *in vitro*

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Abstract

Benzothiadiazole (BTH) is a structural analogue of salicylic acid (SA) which is widely recognized for its role in elicitation of systemic acquired resistance in a broad range of plant species. Here, BTH was applied to cell cultures of the bulbous ornamental plants *Ornithogalum dubium* and *O. thyrsoides*, showing a strong effect on rates of differentiation and morphogenesis. Morphogenic cell clusters in liquid Murashige and Skoog (MS) medium containing 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) were used for all treatments. The calluses were washed thoroughly and activated with increasing concentrations of BTH. Following the induction, calli were grown on a solid MS medium without growth regulators (MS) or on a comparable media with NAA and BAP (M-206). The calli treated with BTH displayed a dose dependent increase in formation of meristematic centres followed by enhanced shoot formation compared to controls. Microscopic analyses revealed increased differentiation to cell organelles and a strengthening of the cell wall. A stronger response to BTH was observed in MS than in M-206 medium. A similar effect on calli differentiation was obtained by three weeks darkness followed by light exposure. The dark/light positive effect on differentiation was further augmented by BTH in a synergistic fashion. It is suggested that BTH enhances the rates of morphogenesis in *Ornithogalum* cultures by triggering a plant regulator-like activity.

Additional key words: benzothiadiazole, 6-benzylaminopurine, micropropagation, morphogenesis, 1-naphthaleneacetic acid.

Introduction

Ornithogalum is a genus of perennial plants recently reclassified in the family *Hyacinthaceae* (Meerow 2002, Chase 2004). About 200 species are known, originating from Africa, Europe, and Asia, some of which produce long-lasting cut flowers. The most commonly grown species for the cut flower industry are *O. dubium*, *O. thyrsoides, O. arabicum*, and *O. saundersiae* whereas others, such as *O. umbellatum*, *O. nutans*, and *O. pyramidale*, are used for gardening (Griesbach *et al.* 1993, Littlejohn and Blomerus 1997). One of the factors limiting the commercial production is high sensitivity to both viral and bacterial pathogens (Byther and Chastagner 1993, Luria *et al.* 2002) which is associated with the vegetative propagation from bulbs. Alternatively, tissue culture techniques for propagation of some *Ornithogalum* species including *O. dubium* and *O. thyrsoides* have been successfully applied in the past (Hussey 1976, Ziv and Lilien-Kipnis 2000, Kariuki and Kako 2003, Roh *et al.* 2007, Ozel *et al.* 2008). Still, keeping disease-free propagation material and increasing the propagation rates of desired genotypes are a continuous challenge in *Ornithogalum* production (Naik and Nayak 2005).

Benzo-1,2,3-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), also known as *Bion*® (*Syngenta,* Basel, Switzerland), is known to act as a functional analogue of salicylic acid (SA) in plants. It was first reported as an environmentally-friendly crop protectant that imitates SA in activating the plant's defence system (Friedrich *et al.* 1996, Louws *et al.* 2001, Goellner and Conrath 2008).

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Abbreviations: BAP - 6-benzylaminopurine; BTH - benzothiadiazole; MS - Murashige and Skoog; MS-206 - MS medium supplemented with NAA and BAP, NAA - 1-naphthaleneacetic acid; PBS - phosphate buffered saline; SA - salicylic acid.
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Although BTH itself has no anti-microbial properties, it was convincingly shown to increase the plant resistance to disease by activating the signal transduction pathway leading to systemically acquired resistance (Gorlach *et al.* 1996, Lawton *et al.* 1996, Gozzo 2003). BTH was found to be effective against viral, bacterial, and fungal plant pathogens (Gorlach *et al.* 1996, Benhamou and Belanger 1998, Cole 1999). The effect of BTH on plant defence response has been studied thoroughly in a variety of crops including: tomato (Amini 2009), cotton (Whan *et al.* 2008), cucumber (Liu *et al.* 2008), potato (Navarrea and Mayo 2004), mango (Zhu *et al.* 2008), soybean (Faessel *et al.* 2008), red clover (Kusumoto *et al.* 2007), wheat (Vechet *et al.* 2009), peach (Liu *et al.* 2005), *Zantedeschia* (Luzzatto *et al.* 2007a,b), and others. While searching for resistance traits in tissue culture of *Ornithogalum* spp. (Golan *et al.* 2010), BTH was found to enhance micropropagation rates of the plant. Hence, it was hypothesized that BTH may also affect morphogenesis in tissue culture. Two species of ornamental bulb plants, *O. dubium* and *O. thyrzoides*, served to demonstrate the effects of different BTH concentrations on early developmental stages.

Materials and methods

Chemicals were purchased from *Sigma-Aldrich* (St. Louis, MI, USA) unless specified. Media used for the tissue culture were purchased from *Duchefa*, (Haarlem, The Netherlands) unless otherwise mentioned.

Ornithogalum dubium Houtt. and *O. thyrsoides* Jacq. were used for callus initiation from basal parts of axenic leaf segments. Initial morphogenic cell clusters of both plant species were generated from the calli by gently separating it into roughly 2 mm fragments. The fragments were placed in 20 cm^3 of liquid Murashige and Skoog (1962; MS) medium supplemented with 0.1 mg dm^{-3} 1-naphthaleneacetic acid (NAA), 2 mg dm^{-3} 6-benzyl-aminopurine (BAP) and 3 % sucrose (M-206) in 120 cm^3 Erlenmeyer flasks. The pH of the media was adjusted to 5.6 - 5.7 before autoclaving at 121 °C for 20 min (Cohen *et al.* 2004). The nearly homogenous cell clusters developed were incubated on a rotary shaker at 100 rpm, 25 ºC in the dark.

Two weeks old cell clusters were washed and transferred to liquid MS medium containing BTH dissolved in double distilled water (DDW) to obtain final concentrations of: 0 (control), 5, 50, and 100 mg dm^{-3}. BTH solutions were filtered through 0.22 µm filter (*Millipore*, Carrigtwohill, Ireland) prior to use. Flasks were placed on a rotary shaker at 100 rpm, 25 ºC for additional 48 h in the dark. Following the induction treatment, calli were sub-cultured in Petri dishes (55 mm diameter, 12 mm deep) on solid media (6 g dm^{-3} agar) with 3 % sucrose. Two solid media were used: standard MS salts, and MS salts supplemented with 0.1 mg dm^{-3} NAA and 2 mg dm^{-3} BAP (M-206) (Cohen *et al.* 2004). A period of darkness followed by irradiance was shown to induce embryo formation and morphogenesis in sugar cane (Garcia *et al.* 2007), lily (Mori *et al.* 2005), sorghum (Pola *et al.* 2007), *etc*. We found previously that 3 weeks of darkness followed by 14-h photoperiod induced strong morphogenesis of *O. dubium* calli (unpublished results). Accordingly, the effect of three weeks of darkness followed by 14-h photoperiod only was compared to that of BTH alone and with combined effects of both. All plates were incubated in a growth chamber at temperature of 25 ± 2 ºC and irradiance of 60 µmol m^{-2} s^{-1} provided by fluorescent light bulbs. In all treatments, plates were photographed using a digital camera (*PowerShot SX200 IS*, *Canon*, Oita, Japan) and meristematic centers and shoot initiations developing on each initial callus were analyzed using the digital images.

Samples of *O. dubium* calli treated with BTH were taken from MS or M-206 medium after 24 or 91 d. Tissue was fixed in 3.5 % glutaraldehyde in phosphate buffered saline (PBS), rinsed and postfixed in 1 % OsO_4 in PBS. After several washes in PBS, the tissue was stained in the block with uranyl acetate. The samples were then dehydrated by passing them through ethanol series and acetone. After dehydration, the samples were embedded in epoxy resin *Agar100* (*Agar Scientific*, Cambridge, UK). Ultrathin sections were cut, mounted on formvar/carbon grids, contrasted with uranyl acetate and lead citrate, and examined in a *Tecnai* G^2 *Spirit* transmission electron microscope (TEM; *FEI Company*, *Philips*, Eindhoven, The Netherlands). The images were taken using *MegaView III* (*Olympus*, Münster, Germany) camera and analysed using software *SIS* (*Soft Imaging System*, Münster, Germany).

The plates were arranged in a randomized blocks design and analyzed at 24, 42, 72, and 120 d after the induction. Experiments were repeated twice with similar trends. One-way *ANOVA* using by *PRISM 3.02* software (*GraphPad*, San Diego, CA) was used for statistical analysis. Where *ANOVA* yielded significant ($P < 0.05$) differences, *post hoc* analysis was performed using Tukey-Kramer multiple comparisons test.

Results and discussion

Meristematic centers appeared on the BTH pre-treated *O. dubium* calli 21 d after the transfer of cell clusters from liquid to solid MS medium. It took 35 d for the same process to occur in the non-treated control. Shoot regeneration was visible within 63 d in the BTH treatment, a week earlier than in the control. The number

of shoots in the 50 mg dm^{-3} BTH treatment at 72 and 120 d post BTH application was considerably higher than in the control ($P < 0.001$; Table 1, Fig. 1*A*). The effect of BTH on differentiation was also studied in M-206 medium (the common medium for *O. dubium* micropropagation; Cohen *et al.* 2005). Significant increase in number of shoots was observed at 50 mg dm^{-3} BTH (Table 1, Fig. 1*B*) while 100 mg dm^{-3} BTH was less effective.

To further test the effect of BTH on differentiation, the same set of experiments were conducted in *O. thyrisoides*. Here, meristematic centers appeared 5 d after BTH treatment and transfer of the calli to solid medium, *i.e.*, 9 d before their emergence in the control treatment. Shoot regeneration in the BTH treated and control calli appeared after 35 and 41 d, respectively. After 72 d, the number of shoots in the calli treated with 50 mg dm^{-3} BTH was more than double in comparison with control ($P < 0.001$). Here again, BTH was found to induce differentiation and appearance of meristematic

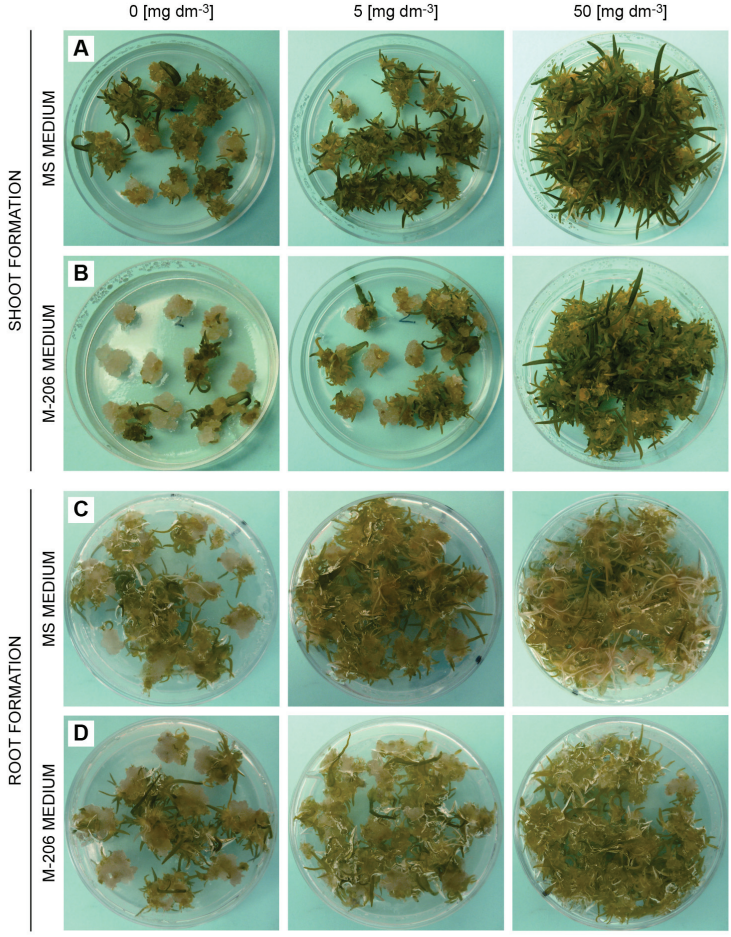

Fig. 1. Effect of increasing concentration of BTH on the regeneration of *Ornithogalum dubium*. Calli were treated with 0 (control), 5, and 50 mg dm^{-3} BTH for 2 d in the dark. Following treatment, the calli were transfered to MS or M-206 medium. Shoot and root formation was observed after 120 d.

Table 1. Effect of BTH on the development of meristematic centers and shoot regeneration of *O. dubium* calli on MS and M-206 medium. Means ± SE, $n = 100$, ** and *** - significantly different at $P < 0.01$ and 0.001, respectively.

BTH [mg dm^{-3}]	MS number of centers [callus^{-1}]		number of shoots [callus^{-1}]		M-206 number of centers [callus^{-1}]		number of shoots [callus^{-1}]	
	24 d	42 d	72 d	120 d	24 d	42 d	72 d	120 d
0	0.51±0.63	3.78±0.29	1.96±0.22	4.95±0.40	0.02±0.01	0.76±0.07	0.15±0.06	1.78±0.30
5	0.57±0.12	2.72±0.31	1.26±0.19	5.06±0.61	0.06±0.02	1.84±0.28**	0.64±0.14^{s}	3.86±0.63
50	2.83±0.32***	9.41±0.54***	5.66±0.50***	13.94±1.20***	0.53±0.11***	4.62±0.40***	1.44±0.22***	11.46±1.17***
100	0.77±0.16	6.22±0.63***	4.36±0.50***	18.47±1.50***	0.06±0.02	0.44±0.08	0.23±0.12	6.48±1.92**

Table 2. Effect of BTH on the development of meristematic centers and shoot regeneration of *O. thyrosides* calli on MS and M-206 media. Means ± SE, $n = 100$, *, *** - significantly different at $P < 0.05$ and 0.001, respectively.

BTH [mg dm^{-3}]	MS number of centers [callus^{-1}]		number of shoots [callus^{-1}]		M-206 number of centers [callus^{-1}]		number of shoots [callus^{-1}]	
	24 d	42 d	72 d	120 d	24 d	42 d	72 d	120 d
0	0.21±0.05	0.96±0.12	1.50±0.14	2.49±0.21	0.02±0.04	1.03±0.18	1.06±0.11	2.91±0.29
5	0.30±0.16	1.37±0.16	1.49±0.14	3.51±0.32	0.20±0.04	1.98±0.19*	1.30±0.13	4.39±0.43***
50	0.27±0.05	1.36±0.15	2.42±0.19***	4.14±0.33***	0.09±0.03	1.75±0.12*	2.06±0.16***	5.03±0.33***
100	0.84±0.14***	2.01±0.24***	2.98±0.37***	5.65±0.52***	0.23±0.06^{ns}	1.72±0.17*	2.10±0.22***	4.47±0.37***

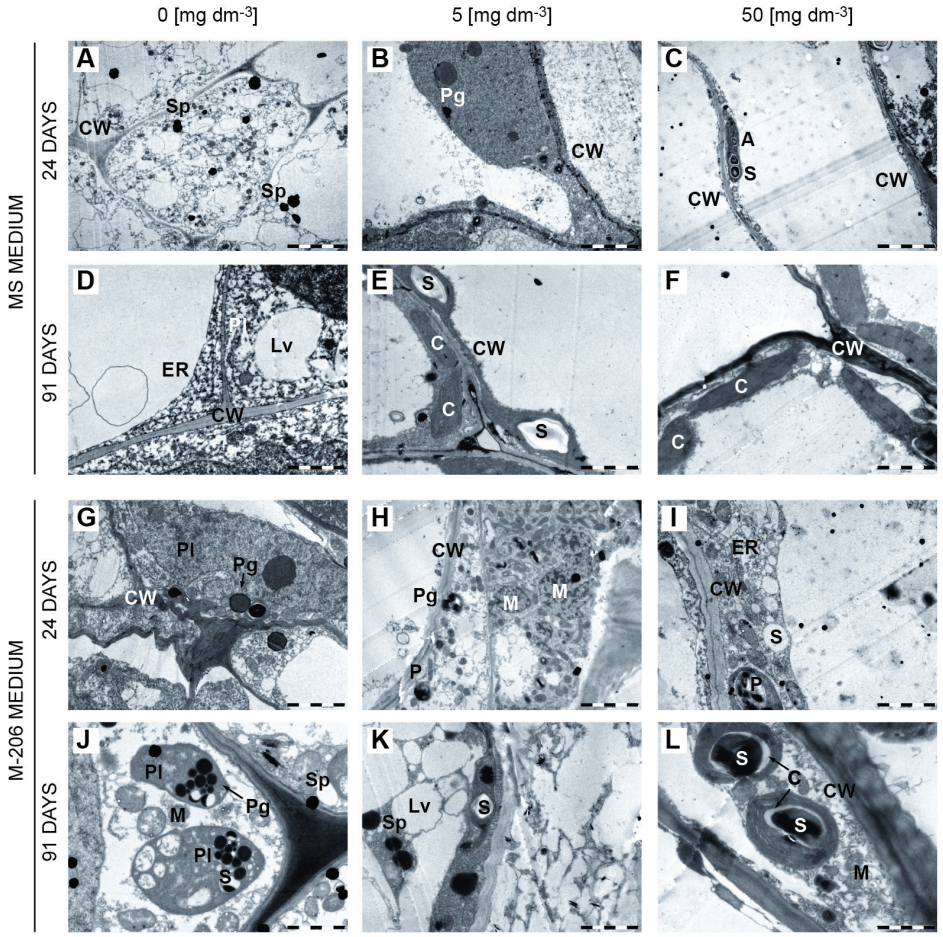

Fig. 2. Electron micrographs of developing *Ornithogalum dubium* calli cells grown on MS medium or M-206 medium and treated with increasing BTH concentrations under 14-h photoperiod. *O. dubium* calli treated with 0, 5, and 50 mg dm^{-3} BTH and transferred to MS medium for 24 d (*A*, *B*, and *C*; *scale bar* = 5 μm) or 91 d (*D*, *E*, and *F*; *scale bar* = 2 μm). *O. dubium* calli treated with the same BTH concentrations and transferred to M-206 medium for 24 d (*G*, *H*, and *I*; *scale bar* = 5 μm) or 91 d (*J*, *K*, and *L*; *scale bar* = 2 μm). A - amyloplast, C - chloroplast, CW - cell wall, ER - endoplasmatic reticulum, Lv - large vesicle, PI - proplastid, Pg - plastoglobulus, S - starch grain, Pp - spherosome.

centers and shoots in the initiative calli (Table 2). The effect of BTH on differentiation of *O. thyrsoides* calli grown on M-206 medium was also examined (Table 2).

While the effect of BTH application on differentiation was clearly visible for both *Ornithogalum* species and in both media, differences were observed in the development of the calli. The overall development of *O. dubium* on MS medium was significantly better than on M-206 (Fig. 1*A*,*B*) while *O. thyrsoides* development was similar on the two media (Table 2). *O. dubium* responded to the highest dose of BTH used here (100 mg dm^{-3}) more on MS than on M-206 medium. In *O. thyrsoides*, 100 mg dm^{-3} BTH mostly enhanced differentiation regardless of the medium. It is hence apparent that

different species, with probably different content of endogenous phytohormones, showed different responses to application of growth regulators in the medium. Indeed, the early application of NAA and BAP to the initial cell culture of *O. dubium*, prior to the transfer to the solid medium, was sufficient to initiate differentiation. In *O. thyrsoides*, the presence of growth regulators in the solid medium did not affect calli development or its response to BTH. In practice, to optimize calli differentiation and regeneration using BTH, each plant system needs to be analyzed individually to establish a protocol for BTH application.

Dose-dependent relationships were observed, using TEM, between the differentiation to cell organelles and BTH concentration in calli grown on MS medium. Higher BTH concentrations induced the formation of amyloplasts, chloroplasts, and starch grains. *O. dubium* calli treated with 5 mg dm^{-3} BTH revealed plastids containing plastoglobuli. 50 mg dm^{-3} BTH induced fully developed amyloplasts with starch grains after 24 d, while at the same time point, calli in the control treatment were still at an early stage of differentiation (Fig. 2*A*-*C*). After 91 d, calli treated with 50 mg dm^{-3} BTH revealed fully mature chloroplasts with an internal network of grana and thylakoids, while the control calli had differentiated into an early stage of proplastids (Fig. 2*D*-*F*). The microscopic observation of *O. thyrsoides* on MS medium revealed similar trend, specifically accumulation of starch grains and prominent cell wall appositions that appeared mainly in the 50 mg dm^{-3} BTH-induced calli (data not shown).

Accumulation of starch in calli prior to shoot initiation is well known phenomenon. It was shown, *e.g.*, in tobacco callus culture (Thorpe and Murashige 1968), where accumulation of starch was well organized and cells that accumulated high starch content differentiated into meristemoids and shoot primordia. During outgrowth of the primordia into shoots, starch was completely utilized (Thorpe and Meier 1972). Thus, it is suggested that also here the early accumulation of starch and formation of starch grains in the 5 and 50 mg dm^{-3} BTH treated calli can promoted chloroplast development and formation of shoots.

Micrographs of *O. dubium* grown on M-206 medium (Fig. 2*G*-*L*) revealed similar trends to those observed on MS medium (Fig 2*A*-*F*). An enhanced differentiation at the plant-cell level was demonstrated for the BTH-induced calli. After 91 d, the control calli revealed clear plastids containing plastoglubuli and starch while the BTH induced tissue revealed fully developed chloroplasts with thylakoids and large starch grains (Fig 2*J*-*L*). The results also support earlier observations on the effect of BTH on thickening and probably strengthening of the cell wall shown previously in several systems including wheat (Gorlach *et al.* 1996) and tomato (Benhamou and Belanger 1998). While these reactions are certainly related to the enhanced resistance to plant pathogens achieved by the application of BTH, they also point to hormone-like activity of BTH.

The effect of BTH was further examined on root establishment in the *O. dubium* calli, showing an enhanced formation of roots at 50 mg dm^{-3} BTH (Table 3, Fig. 1*C*,*D*). The root development was more prominent on MS medium (Fig. 1*C*) than on M-206 medium (Fig. 1*D*). Similar to the effect on the shoots, the presence of NAA and BAP in solid M-206 medium negatively interfered with the development of roots in the *O. dubium* calli. Furthermore, BTH induced a slight increase in fresh mass and callus basal area at early stages of development (data not shown); this effect, however, disappeared at later stages. Fresh mass and basal area of calli after 120 d in culture reached the same values in both BTH treated and control cultures (data not shown). This was true for both *Ornithogalum* species and the two growth media. The absence of any measurable increase in biomass at the later stages of growth suggests that the significant difference in regeneration rates following application of BTH is a consequence of altered morphogenesis rate of the tissue. BTH application switched earlier the differentiation of the calli from a mass of undifferentiated cells towards morphogenesis giving rise to more meristematic centers, shoots, and roots, but not to an overall higher production of biomass.

Table 3. Effect of BTH and darkness on number of roots per *O. dubium* callus on MS and M-206 media at 14-h photoperiod for whole 120-d period or firstly grown in darkness (21 d) and then at 14-h photoperiod. Means ± SE, n = 100, *** - significantly different at $P < 0.001$.

BTH [mg dm^{-3}]	MS		M-206	
		darkness		darkness
0	0.59±0.11	1.16±0.26	0.07±0.04	0.01±0.01
5	0.36±0.12	1.50±0.46	0.10±0.06	0.00±0.00
50	4.28±0.63***	2.64±0.48	0.78±0.15***	0.58±0.36***
100	1.58±0.33	1.40±0.44	0.05±0.02	0.40±0.20

In previous reports, BTH was suggested to act as one of the known plant signalling molecules including SA, jasmonic acid, and abscisic acid. For example, BTH induced somatic embryogenesis receptor kinase (*SERK1*) gene in rice (Hu *et al.* 2005). The constitutive over-expression of this gene enhanced shoot regeneration rate alongside with resistance to fungal infection (Hu *et al.* 2005). A similar phenomenon was observed in *Dactylis glomerata* where *SERK1* homologue was found to closely follow the development of cells competent to form somatic embryos (Omid *et al.* 2009). It may thus be suggested that here the application of BTH to *Ornithogalum* involves induction of *SERK1* and consequently shoot regeneration. This can also lead to thickening of the cell walls of treated callus observed here and to the well documented enhanced resistance to fungal infection. In *Lemna*, BTH induced flowering (Endo *et al.* 2009). Thus, BTH, mainly recognized for its capability to induce systemic acquired resistance in plants

Table 4. Effect of BTH and darkness on the development of meristematic centers and shoot regeneration of *O. dubium* calli on MS and M-206 media. Firstly, the calli were grown in the darkness for 21 d and then transferred to 14-h photoperiod. Means ± SE, $n = 100$, ** and *** - significantly different at $P < 0.01$ and 0.001, respectively.

BTH [mg dm^{-3}]	MS			M-206		
	number of centers [callus^{-1}]	number of shoots [callus^{-1}]		number of centers [callus^{-1}]	number of shoots [callus^{-1}]	
	42 d	72 d	120 d	42 d	72 d	120 d
0	0.37±0.12	0.20±0.09	15.25±1.21	0.00±0.00	0.00±0.00	0.50±0.77
5	0.87±0.24	1.60±0.49	15.30±2.46	0.00±0.00	0.00±0.00	0.55±0.19
50	9.00±0.87***	7.77±1.01***	30.70±1.96***	0.00±0.00	0.15±0.11	10.90±2.79***
100	7.47±0.89***	4.46±0.50**	26.07±2.22***	0.30±0.11***	1.45±0.44***	14.00±2.24***

(Gozzo 2003, Kusumoto *et al.* 2007, Umemura *et al.* 2009), exhibits a broader range of activities including morphogenesis in the genus *Ornithogalum*.

Light is invariably essential for the growth of green shoots and plantlets, however, unorganised cell and tissue clusters can frequently be successfully grown in darkness (George *et al.* 2008). A short period of darkness sometimes promotes shoot morphogenesis. Such an example are adventitious buds of *Picea pungens* whose formation on swollen needle primordia was found to require at least 8 d of darkness (Mission *et al.* 1982). Positive effects of continuous darkness followed by suitable photoperiod on plant differentiation have also been described in geophytes (Dimech *et al.* 2007). Although the storage organs in geophytes develop in the ground, exposure to light may play a role in inducing differentiation. When *Ornithogalum* calli were grown in the darkness for 3 weeks and then transferred into a 14-h photoperiod, the formation of meristematic centres and shoots was enhanced. The process was not immediate, the dark period firstly inhibited differentiation compared to control calli that was cultured at 14-h photoperiod from the beginning. Nevertheless, after 120 d, the initial dark treatment increased the number of shoots, an effect that was further promoted by BTH (Table 4). After 120 d, the number of shoots in calli grown under combined treatment was higher in comparison to calli grown steadily under 14-h photoperiod. Moreover, the shoots of the controls appeared pale and relatively thin while BTH activated not only shoot regeneration but also induced vigorous shoots. In both media, 3 weeks of darkness followed by 14-h photoperiod in a combination with BTH significantly increase the number of shoots and improved their overall appearance. A similar effect was observed in *O. thyrsoides* (data not shown).

Unlike the positive effect on shoot development, 3 week darkness followed by 14-h photoperiod reduced the number of roots produced on *O. dubium* calli relative to 14-h photoperiod during the whole experiment. The results presented here support an activating role for BTH in regeneration of *Ornithogalum* plantlets in tissue culture. The effects were dose dependent and the concentration of BTH and medium type were crucial for a positive response in both species. The efficacy of BTH treatment to induce calli differentiation was higher for *O. dubium* than for *O. thyrosoides*. This conforms to an overall better regenerability of *O. dubium* in tissue culture. The results support previous records showing that regenerability varies with genotypes and even among cells within the same plant (Murashige and Huang 1985). BTH was originally developed as an immunizing agent to sensitize various crops against pathogen infection through the activation of the SA pathway (Gorlach *et al*. 1996, Cole 1999, Gozzo 2003, Herman *et al*. 2008). It is now accepted that SA is involved not only in defence responses in various plants but also in seed germination, growth and flowering (Vlot *et al*. 2009). In cell cultures of *Capsicum chinense*, SA stimulated secondary metabolite production (Gutierrez-Carbajal *et al*. 2010). However, such functions were not described for the SA analogue BTH. Further work is required to establish and elucidate the mechanism underlying the effects of synthetic BTH on differentiation and morphogenesis. As the genus *Ornithogalum* is closely related to other major petaloid monocots such as the *Liliaceae* and *Amaryllidaceae*, application of BTH may also improve micropropagation of other valuable ornamental crops.

References

Amini, J.: Induced resistance in tomato plants against *Fusarium* wilt invoked by nonpathogenic *Fusarium*, chitosan and Bion. - Plant Pathol. J. **25**: 256-262, 2009.

Benhamou, N., Belanger, R.: Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum f.* sp. *radicis-lycopersici* in tomato. - Plant Physiol. **118**: 1203-1212, 1998.

Byther, R.S., Chastagner, G.A.: Diseases. - In: De Hertogh, A., Le Nard, M. (ed.): The Physiology of Flower Bulbs. Pp. 71-100. Elsevier Science, Amsterdam 1993.

Chase, M.: Monocot relationships: an overview. - Amer. J. Bot. **91**: 1645-1655, 2004.

Cohen, A., Lipsky, A., Arazi, T., Ion, A., Stav, R., Sandler-Ziv, D., Fintea, C., Gaba, V., Gera, A.: Particle bombardment-mediated transformation of *Ornithogalum dubium* for *Ornithogalum* mosaic virus resistance. - Acta Hort. **673**: 183-190, 2005.

Cohen, A., Lipsky, A., Arazi, T., Ion, A., Stav, R., Sandler-Ziv, D., Pintea, C., Barg, R., Salts, Y., Shabtai, S., Gaba, V., Gera, A.: Efficient genetic transformation of *Lilium longiflorum* and *Ornithogalum dubium* by particle acceleration followed by prolonged selection in liquid medium. - Acta Hort. **651**: 131-138, 2004.

Cole, D.: The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance, against bacterial and fungal diseases of tobacco. - Crop Protect. **18**: 267-273, 1999.

Dimech, A., Cross, R., Ford, R., Taylor, P.: Micropropagation of gymea lily (*Doryanthes excelsa Correa*) from new South Wales, Australia. - Plant Cell Tissue Organ Cult. **88**: 157-165, 2007.

Endo, J., Takahashi, W., Ikegami, T., Beppu, T., Tanaka, O.: Induction of flowering by inducers of systemic acquired resistance in the *Lemna* plant. - Biosci. Biotechnol. Biochem. **73**: 183-185, 2009.

Faessel, L., Nassr, N., Lebeau, T., Walter, B.: Effects of the plant defence inducer, acibenzolar-*S*-methyl, on hypocotyl rot of soybean caused by *Rhizoctonia solani* AG-4. - J. Phytopathol. **156**: 236-242, 2008.

Friedrich, L., Lawton, K.A., Ruess, W., Masner, P., Specker, N., Rella, M. G., Meier, B., Dincher, S., Staub, T., Uknes, S., Metraux, J.P., Kessmann, H., Ryals, J.: A benzo-thiadiazole derivative induces systemic acquired resistance in tobacco. - Plant J. **10**: 61-70, 1996.

Garcia, R., Cidade, D., Castellar, A., Lips, A., Magioli, C., Callado, C., Mansur, E.: *In vitro* morphogenesis patterns from shoot apices of sugar cane are determined by light and type of growth regulator. - Plant Cell Tissue Organ Cult. **90**: 181-190, 2007.

Goellner, K., Conrath, U.: Priming: it's all the world to induced disease resistance. - Eur. J. Plant Pathol. **121**: 233-242, 2008.

Golan, A., Kerem, Z., Tun, O.M., Luzzatto, T., Lipsky, A., Yedidia, I.: Combining flow cytometry and *gfp* reporter gene for quantitative evaluation of *Pectobacterium carotovorum* ssp *carotovorum* in *Ornithogalum dubium* plantlets. - J. appl. Microbiol. **108**:1136-1144, 2010.

George, E.F., Hall, M.A., De Klerk, G.D. (ed.): Plant Propagation by Tissue Culture. Pp. 423-464. - Springer, London 2008.

Gorlach, J., Volrath, S., Beiter, G.K., Hengy, G., Beckhove, U., Kogel, K., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., Ryals, J.: Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. - Plant Cell **8**: 629-643, 1996.

Gozzo, F.: Systemic acquired resistance in crop protection: from nature to a chemical approach. - J. Agr. Food Chem. **51**: 4487-4503, 2003.

Griesbach, R.J., Meyer, F., Koopowitz, H.: Creation of new flower colors in *Ornithogalum* via interspecific hybridization. - J. amer. Soc. hort. Sci. **118**: 409-414, 1993.

Gutierrez-Carbajal, M.G., Monforte-Gonzalez, M., Miranda-Ham, M.D., Godoy-Hernandez, G., Vazquez-Flota, F.: Induction of capsaicinoid synthesis in *Capsicum chinense* cell cultures by salicylic acid or methyl jasmonate. - Biol. Plant. **54**: 430-434, 2010.

Herman, M., Davidson, J., Smart, C.: Induction of plant defence gene expression by plant activators and *Pseudomonas syringae* pv. *tomato* in greenhouse-grown tomatoes. - Phytopathology **98**: 1226-1232, 2008.

Hu, H., Xiong, L., Yang, Y.: Rice *SERK1* gene positively regulates somatic embryogenesis of cultured cell and host defence response against fungal infection. - Planta **222**: 107-117, 2005.

Hussey, G.: Plantlet regeneration from callus and parent tissue in *Ornithogalum-thyrsoides*. - J. exp. Bot. **27**: 375-382, 1976.

Kariuki, W., Kako, S.: Micropropagation of *Ornithogalum saundersiae* Bak. - Acta Hort. **624**: 521-526, 2003.

Kusumoto, D., Goldwasser, Y., Xie, X., Yoneyama, K., Takeuchi, Y., Yoneyama, K.: Resistance of red clover (*Trifolium pratense*) to the root parasitic plant *Orobanche minor* is activated by salicylate but not by jasmonate. - Ann. Bot. **100**: 537-544, 2007.

Lawton, K.A., Friedrich, L., Hunt, M., Weymann, K., Delaney, T., Kessmann, H., Staub, T., Ryals, J.: Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. - Plant J. **10**: 71-82, 1996.

Littlejohn, G.M., Blomerus, L.M.: Evaluation of *Ornithogalum* genebank accessions for some characteristics of importance for breeding cut flowers or pot plants. - Genet. Resour. Crop Evol. **44**: 227-234, 1997.

Liu, C., Ruan, Y., Lin, Z., Wei, R., Peng, Q., Guan, C., Ishii, H.: Antagonism between acibenzolar-S-methyl-induced systemic acquired resistance and jasmonic acid-induced systemic acquired susceptibility to *Colletotrichum orbiculare* infection in cucumber. - Physiol. mol. Plant Pathol. **72**: 141-145, 2008.

Liu, H.X., Jiang, W.B., Bi, Y., Luo, Y.B.: Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by *Penicillium expansum* and enhances activity of fruit defence mechanisms. - Postharvest Biol. Technol. **35**: 263-269, 2005.

Louws, F.J., Wilson, M., Campbell, H.L., Cuppels, D.A., Jones, J.B., Shoemaker, P.B., Sahin, F., Miller, S.A.: Field control of bacterial spot and bacterial speck of tomato using a plant activator. - Plant Dis. **85**: 481-488, 2001.

Luria, G., Watad, A.A., Zhedek, Y.C., Borochov, A.: Growth and flowering of *Ornithogalum dubium*. - Acta Hort. **570**: 113-119, 2002.

Luzzatto, T., Golan, A., Yishay, M., Bilkis, I., Ben-Ari, J., Yedidia, I.: Priming of antimicrobial phenolics during induced resistance response towards *Pectobacterium carotovorum* in the ornamental monocot calla lily. - J. Agr. Food Chem. **55**: 10315-10322, 2007a.

Luzzatto, T., Yishay, M., Lipsky, A., Ion, A., Belausov, E., Yedidia, I.: Efficient, long-lasting resistance against the soft rot bacterium *Pectobacterium carotovorum* in calla lily provided by the plant activator methyl jasmonate. - Plant Pathol. **56**: 692-701, 2007b.

Meerow, A.W.: The new phylogeny of the Lilioid mono-cotyledons. - Acta Hort. **570**: 31-45, 2002.

Mission, J., Coumans, M., Wirgot, G., Gospar, T.: [Induction of adventitious buds on *Picea pungens in vitro*.] - Z. Pflanzenphysiol. **107**: 161-167, 1982 [In French.].

Mori, S., Adachi, Y., Horimoto, S., Suzuki, S., Nakano, M.: Callus formation and plant regeneration in various *Lilium* species and cultivars. - *In Vitro* cell. dev. Biol. Plant **41**: 783-788, 2005.

Murashige, T., Huang, L.: Organogenisis *in Vitro*: Sructural, Physiological, and Biochemical Aspects. - International Rice Research Institute, Manila 1985.

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

Naik, P.K., Nayak, S.: Different modes of plant regeneration and factors affecting *in vitro* bulblet production in *Ornithogalum virens*. - Sci. Asia **31**: 409-414, 2005.

Navarrea, D.A., Mayo, D.: Differential characteristics of salicylic acid-mediated signaling in potato. - Physiol. mol. Plant Pathol. **64**: 179-188, 2004.

Omid, K., Behzad, A., Aghil M.P.: Molecular aspects of somatic-to-embryogenic transition in plants. - J. chem. Biol. **2**: 177-190, 2009.

Ozel, C.A., Khawar, K.M., Karaman, S., Ates, M.A., Arslan, O.: Efficient *in vitro* multiplication in *Ornithogalum ulophyllum* Hand.-Mazz. from twin scale explants. - Sci. Hort. **116**: 109-112, 2008.

Pola, S., Saradamani, N., Ramana, T.: Enhanced shoot regeneration in tissue culture studies of *Sorghum bicolor*. - J. Agr. Technol. **3**: 275-286, 2007.

Roh, M.S., Lee, A.K., Suh, J.K.: Induction of bulb maturity of *Ornithogalum thyrsoides*. - Sci. Hort. **114**: 138-141, 2007.

Thorpe, T., Meier, D.: Starch metabolism, respiration, and shoot formation in tobacco callus cultures. - Physiol Plant **27**: 365-369, 1972.

Thorpe, T., Murashige, T.: Starch accumulation in shoot-forming tobacco callus cultures. - Science **160**: 421-422, 1968.

Umemura, K., Satou, J., Iwata, M., Uozumi, N., Koga, J., Kawano, T., Koshiba, T., Anzai, H., Mitomi, M.: Contribution of salicylic acid glucosyltransferase, OsSGT1, to chemically induced disease resistance in rice plants. - Plant J. **57**: 463-472, 2009.

Vechet, L., Burketova, L., Sindelarova, M.: A comparative study of the efficiency of several sources of induced resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat under field conditions. - Crop Prot. **28**: 151-154, 2009.

Vlot, A., Dempsey, D., Klessig, D.: Salicylic acid, a multifaceted hormone to combat disease. - Annu. Rev. Phytopathol. **47**: 177-206, 2009.

Whan, J., Dann, E., Smith, L., Aitken, E.: Acibenzolar-S-methyl-induced alteration of defence gene expression and enzyme activity in cotton infected with *Fusarium oxysporum* f. sp. *vasinfectum*. - Physiol. mol. Plant Pathol. **73**: 175-182, 2008.

Zhu, X., Cao, J., Wang, Q., Jiang, W.: Postharvest infiltration of BTH reduces infection of mango fruits (*Mangifera indica* L. cv. Tainong) by *Colletotrichum gloeosporioides* and enhances resistance inducing compounds. - J. Phytopathol. **156**: 68-74, 2008.

Ziv, M., Lilien-Kipnis, H.: Bud regeneration from inflorescence explants for rapid propagation of geophytes *in vitro*. - Plant Cell Rep. **19**: 845-850, 2000.