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ORGANOGENESIS OF *CYMBIDIUM* ORCHID USING ELICITORS

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Abstract: Elicitors are substances that induce protective responses in plants. In this study, methyl jasmonate (Me-JA) and lysozyme elicitation on PLBs culture of *Cymbidium insigne in vitro* was investigated. Elicitation by 0.1 mg/l Me-JA enhanced maximum PLB, shoot and root formation. The effects of lysozyme under white fluorescent tube, results indicated that every concentrations of lysozyme induced PLB, shoot and root formation and 0.1 mg/l lysozyme enhanced maximum formation of PLB, shoot and root compare with control. Lysozyme is known to play a vital role in medical industry and the present study firstly used lysozyme, as a plant growth regulator in *Cymbidium* tissue culture.

Keywords: lysozyme, methyl jasmonate, protocorm-like body, plant growth regulator, in vitro

Introduction

Elicitors are substances that induce protective responses in plants. In the beginning of their research era, they were alternatively called inducers, but because of the broad interpretation of this term, the term elicitors are now commonly accepted. It is well established that, upon the challenge by biotic or abiotic elicitors, plants respond with an array of defenses including the accumulation of secondary metabolites [DORNENBURG, 2004]. The method of elicitor-induced resistance to diseases in plants is characterized by a number of essential advantages: ecological safety, because the method is based on induction of the native immune potential of the host plant rather than on suppression of phytopathogens, a systemic and prolonged protective effect, involvement of multiple defense systems in induced resistance, which makes adaptation of pathogens to protected plants nearly impossible induction of nonspecific resistance to the number of fungi, bacteria, viruses, nematodes, etc. Plant cell culture has recently received a lot of attention as an effective technology for the production of valuable secondary metabolites. Plant cell cultures produce higher quantities of secondary metabolites, often with different profiles compared to their parent plants. A key factor to secondary metabolite production in plant cultures, however, is by elicitation among which treatment with jasmonate or methyl jasmoic is widely used [YUN-SOO & al. 2004; SEE & al. 2011; KOO & HOWE, 2009] and has been applied in orchid tissue cultures [SHIMASAKI & al. 2003; TEIXEIRA DA SILVA, 2012, 2012a]. However, whether it can be used in *Cymbidium kanran* Makino, hybride *Cymbidium* (Twilight Moon 'Day Light'), such as *Cymbidium insigne* has not been reported. *Cymbidiums* are among the most important orchids in horticulture. They are versatile plants, marketed as cut-flowers, buttonholes and as pot plants, producing many

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large, showy, long-lasting flowers [DU PUY & CRIBB, 2007]. Most *Cymbidium* spp. are commercially produced using tissue culture methods. The objective of this study is to investigate the effect of two elicitor; methyl jasmonate (Me-JA) and lysozyme on organogenesis of *Cymbidium insigne in vitro*. Me-JA (methyl jasmonates) is particularly interesting because of the myriad of plant responses associated with its synthesis and presence. Me-JA is emitted by wounded plants [MEYER & al. 2003] and therefore, may represent a means of communication between damaged plants. Lysozymes, also known as muramidase or N-acetylmuramide glycanhydrolase, are glycoside hydrolases. These are enzymes (EC 3.2.1.17) that damage bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins. Lysozyme is abundant in a number of secretions, such as tears, saliva, human milk, and mucus. It is also present in cytoplasmic granules of the polymorphonuclear neutrophils (PMNs). Large amounts of lysozyme can be found in egg white. Lysozyme is widely used in medical industry. This is the first report demonstrating that lysozyme used as a plant growth regulator which increases PLBs, shoot and root formation of *C. insigne*.

Materials and methods

Plant material and explants source

Protocorm-like bodies (PLB) of *Cymbidium insigne* were proliferated in the modified Murashige and Skoog [SHIMASAKI & UEMOTO, 1990] medium by transferred new medium every two months. After excision of PLBs (ca. 3 mm in diameter) into pieces, they were used for explants.

Preparation of elicitors

Two elicitor including lysozyme, filter-sterilized (Wako Pure Chemical Industries, Ltd., Japan) with the concentration of 0 (control), 0.1, 1, 10 and 100 mg/l and also methyle jasmonate (purchased from Sigma) with the concentration of 0 (control), 0.1, 1, 10 and 100 mg/l were mixed with sterilized water used as aqueous solution (50 mg).

Culture methods

Experiment 1. Pick up single PLB with forceps and dipping into different concentrations of lysozyme aqueous solution for half an hour (30 minutes). After half an hour, PLBs were cultured on modified MS medium for 8 weeks (up to root formation).

Experiment 2. Pick up single PLB with forceps and dipping into different concentrations of lysozyme aqueous solution for one hour (60 minutes). After one hour, PLBs were cultured on modified MS medium for 8 weeks (up to root formation).

Experiment 3. Pick up single PLB with forceps and dipping into different concentrations of Me-JA aqueous solution for half an hour (30 minutes). After half an hour, PLBs were cultured on modified MS medium for 6 weeks (up to root formation).

Experiment 4. Pick up single PLB with forceps and dipping into different concentrations of Me-JA aqueous solution for one hour (60 minutes). After one hour, PLBs were cultured on modified MS medium for 6 weeks (up to root formation).

Modified MS medium supplemented with 412.5 mg/l ammonium nitrate, 950 mg/l potassium nitrate, 20 g/L sucrose and 2 g/L phytagel (Sigma KK, Japan) was adjusted to pH 5.5-5.8 before autoclaving. Jars (250 ml UM culture bottle; As One, Japan) with plastic

caps containing 30 ml of medium were used as culture vessels. Five explants were cultured in each culture vessel and three culture vessels were used for each treatment. All cultures were maintained at 25 ºC with a 16 h photoperiod and irradiance of 54 µmol m^{-2} s^{-1} under white fluorescent tube.

Statistical analysis

Experimental data were collected by counting the number of PLBs, shoot and root; percentage of PLBs, shoot and root; the fresh weight of PLBs were measured. The data were analyzed to a one-way analysis variance (ANOVA) and differences between means were tested using Tukey's honestly significant different test ($P \leq 0.05$).

Results

Experiment 1. In vitro growth of *C. insigne*, PLBs were dipping 30 minutes into lysozyme aqueous solution

The results indicated that PLBs of *C. insigne* were significantly increased fresh weight of PLBs, number of PLBs, shoot and root of lysozyme with modified MS media compare with control. Dipping 30 minutes at lysozyme aqueous solution, 0.1 mg/l lysozyme significantly increased the number of PLBs, shoot and root (Tab. 1). The highest average number of PLBs (7.1 PLBs/explant), the highest average number of shoot (1.1 shoots/explant) and the highest average number of root (0.3 roots/explant) were recorded at 0.1 mg/l lysozyme with modified MS media. The maximum PLB formation rate 100% and the maximum shoot formation rate 53% were observed at 0.1 mg/l lysozyme (Fig. 1A); whereas control had less formation of PLBs (67%) and shoot (20%) after 8 weeks of culture. The maximum root formation rate 33% was observed at 0.1 mg/l or 100 mg/l (Fig. 1B) lysozyme with modified MS media, comparatively control treatment had no root formation. The maximum fresh weight of PLBs (94.4 mg) was observed at 1 mg/l lysozyme with modified MS media.

Tab. 1. In vitro growth of *C. insigne*, PLBs were dipping 30 minutes into different concentrations of lysozyme aqueous solution and cultured on modified MS medium under white fluorescent tube for 8 weeks

Lysozyme (mg/l)	PLB			Shoot		Root	
	No. /explant	Rate (%)	FW (mg)	No. /explant	Rate (%)	No. /explant	Rate (%)
Control	2.6 ± 0.7b	67	41.3 ± 3.7c	0.2 ± 0.2b	20	0	0
0.1	7.1 ± 0.9a	100	86.8 ± 11.4ab	1.1 ± 0.5a	53	0.3 ± 0.2a	33
1	3.6 ± 0.7b	80	94.4 ± 16.8a	0.3 ± 0.3ab	27	0.1 ± 0.2b	13
10	3.3 ± 0.7b	87	57.3 ± 6.9bc	0.3 ± 0.2b	27	0.1 ± 0.2b	13
100	4.1 ± 1.5ab	80	45.2 ± 6.4c	0.7 ± 0.4ab	47	0.3 ± 0.2ab	33

*Value represents means±SE followed by the different letters show significant differences by Tukey HSD test ($P \leq 0.05$)

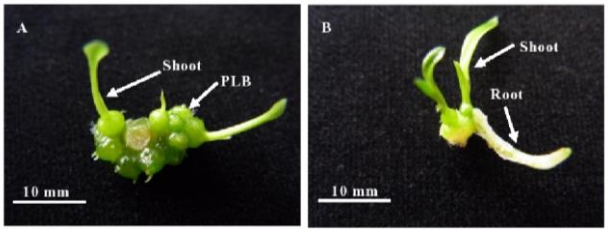

Fig. 1. In vitro growth of *C. insigne*, PLBs were dipping 30 minutes into different concentrations of lysozyme aqueous solution and cultured on modified MS medium under white fluorescent tube for 8 weeks. A: 0.1 mg/l lysozyme; B: 100 mg/l lysozyme.

Experiment 2. In vitro growth of *C. insigne*, PLBs were dipping 60 minutes into lysozyme aqueous solution

Dipping 60 minutes at lysozyme aqueous solution, 1 mg/l lysozyme significantly increased the number of PLBs and fresh weight of PLBs (Tab. 2). The highest PLB formation rate (80%), the highest average number of PLBs (4.9 PLBs/explant), the highest fresh weight of PLBs (79.6 mg) were observed at 1 mg/l lysozyme with modified MS media (Fig. 2A). The highest average number of shoot (0.5 shoots/explant) and the highest shoot formation rate 33% were recorded at 0.1 mg/l or 100 mg/l (Fig. 2B.) lysozyme with modified MS media. The highest root formation rate 33% was recorded which PLBs were dipping 60 minutes at 100 mg/l lysozyme aqueous solution. Comparatively, lowest number of PLBs and lowest fresh weight was observed at control. There was no shoot and root formation observed at control (which PLBs were dipping into water).

Tab. 2. In vitro growth of *C. insigne*, PLBs were dipping 60 minutes into different concentrations of lysozyme aqueous solution and cultured on modified MS medium under white fluorescent tube for 8 weeks

Lysozyme (mg/l)	PLB			Shoot		Root	
	No. /explant	Rate (%)	FW (mg)	No. /explant	Rate (%)	No. /explant	Rate (%)
Control	1.3 ± 0.7b	47	35.3 ± 4.7b	0	0	0	0
0.1	2.3 ± 1.2ab	47	45.9 ± 8.3b	0	0	0	0
1	4.9 ± 1.3a	80	79.6 ± 14.5a	0.5 ± 0.3a	33	0.1 ± 0.2a	13
10	1.7 ± 0.6b	60	45.7 ± 7.1b	0.3 ± 0.4a	20	0.1 ± 0.2a	13
100	2.1 ± 0.8b	53	45.2 ± 6.4b	0.5 ± 0.4a	33	0.3 ± 0.3a	33

*Value represents means±SE followed by the different letters show significant differences by Tukey HSD test (P≤0.05)

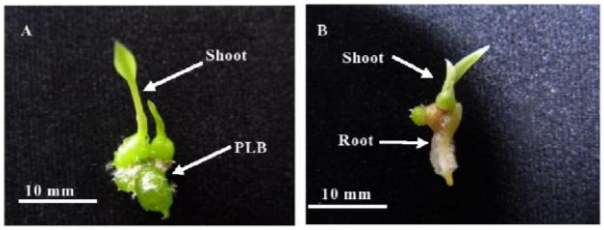

Fig. 2. In vitro growth of *C. insigne*, PLBs were dipping 60 minutes into different concentrations of lysozyme aqueous solution and cultured on modified MS medium under white fluorescent tube for 8 weeks. A: 1 mg/l lysozyme; B: 100 mg/l lysozyme.

Experiment 3. In vitro growth of *C. insigne*, PLBs were dipping 30 minutes into Me-JA aqueous solution

The results indicated that PLBs of *C. insigne* were significantly increased the fresh weight and number of PLBs at Me-JA treatment. Dipping into 30 minutes at Me-JA aqueous solution, 0.1 mg/l Me-JA increased the formation of PLB, shoot and root (Tab. 3). The highest average number of PLBs (4.4 PLBs/explant), the highest average number of shoot (0.4 shoots/explant) and the highest average number of root (0.4 roots/explant) were recorded at 0.1 mg/l Me-JA with modified MS media (Fig. 3). The highest PLBs formation rate 93%, the highest shoot and root formation rate 33% were observed at 0.1 mg/l Me-JA with modified MS media; whereas less formation of PLBs (27%) was observed high concentration of Me-JA (100 mg/l) aqueous solution. 100 mg/l Me-JA aqueous solution had no shoot and root formation observed after 6 weeks of culture. The maximum fresh weight of PLBs (68.3 mg) was observed at 10 mg/l Me-JA with modified MS media.

Tab. 3. In vitro growth of *C. insigne*, PLBs were dipping 30 minutes into different concentrations of Me-JA aqueous solution and cultured on modified MS medium under white fluorescent tube for 6 weeks

Me-JA (mg/l)	PLB			Shoot		Root	
	No. /explant	Rate (%)	FW (mg)	No. /explant	Rate (%)	No. /explant	Rate (%)
Control	2.1 ± 0.7abc	67	44.6 ± 8.2abc	0.3 ± 0.2a	27	0.2 ± 0.2a	20
0.1	4.4 ± 1.0a	93	67.8 ± 10.4ab	0.4 ± 0.3a	33	0.4 ± 0.3a	33
1	2.3 ± 0.8abc	73	38.7 ± 5.9abc	0.2 ± 0.2a	20	0	0
10	4.2 ± 1.0ab	73	68.3 ± 8.6a	0.7 ± 0.4a	27	0	0
100	0.9 ± 0.9c	27	20.9 ± 5.4c	0	0	0	0

*Value represents means±SE followed by the different letters show significant differences by Tukey HSD test (P≤0.05)

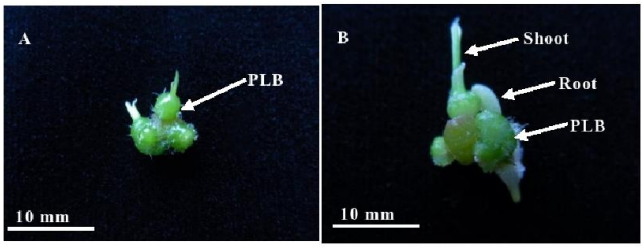

Fig. 3. In vitro growth of *C. insigne*, PLBs were dipping 30 minutes into different concentrations of Me-JA aqueous solution and cultured on modified MS medium under white fluorescent tube for 6 weeks. A: Control; B: 0.1 mg/l Me-JA.

Experiment 4. In vitro growth of *C. insigne*, PLBs were dipping 60 minutes into Me-JA aqueous solution

The results indicated that PLBs of *C. insigne* were significantly increased the fresh weight and number of PLBs at Me-JA treatment. Dipping into 60 minutes at Me-JA aqueous solution, 0.1 mg/l Me-JA with modified MS media increased the formation rate of PLB and shoot (Tab. 4). The highest average number of PLBs (3.6 PLBs/explant), the highest average number of shoot (0.7 shoots/explant) and the maximum fresh weight (58.5 mg) were observed at 0.1 mg/l Me-JA with modified MS media (Fig. 4A). The highest PLB

formation rate 87% and the highest shoot formation rate 40% were recorded at 0.1 mg/l Me-JA with modified MS media; whereas less formation of PLB (33%) was observed high concentration of Me-JA (100 mg/l) aqueous solution. Root formation rate 20% was observed only at 1 mg/l Me-JA aqueous solution after 6 weeks of culture (Fig. 4B).

Tab. 4. In vitro growth of *C. insigne*, PLBs were dipping 60 minutes into different concentrations of Me-JA aqueous solution and cultured on modified MS medium under white fluorescent tube for 6 weeks

Me-JA (mg/l)	PLB			Shoot		Root	
	No. /explant	Rate (%)	FW (mg)	No. /explant	Rate (%)	No. /explant	Rate (%)
Control	3.5 ± 0.9ab	73	51.2 ± 6.8ab	0	0	0	0
0.1	3.6 ± 0.7a	87	58.5 ± 6.8bcd	0.7 ± 0.4a	40	0	0
1	2.1 ± 0.7abc	73	54.4 ± 8.7ac	0.3 ± 0.3a	27	0.2 ± 0.2	20
10	2.3 ± 0.8abc	67	51.1 ± 8.5abd	0.3 ± 0.3a	27	0	0
100	0.4 ± 0.3c	33	20.3 ± 3.5c	0	0	0	0

*Value represents means±SE followed by the different letters show significant differences by Tukey HSD test (P≤0.05)

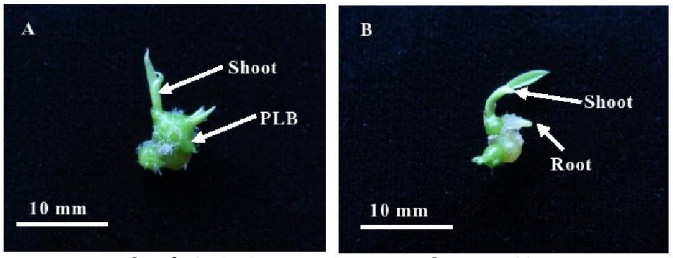

Fig. 4. In vitro growth of *C. insigne*, PLBs were dipping 60 minutes into different concentrations of Me-JA aqueous solution and cultured on modified MS medium under white fluorescent tube for 6 weeks. A: 0.1 mg/l Me-JA; B: 1 mg/l Me-JA.

Discussion

Plant organogenesis *in vitro* is a more controllable and reliable process. *In vitro* propagation of orchids as an option for rapid propagation of commercially valuable cultivars progressed well during the last decades. This is the first report demonstrating lysozyme, work as a plant growth regulator for increasing new PLB, shoot and root formation in *Cymbidium* tissue culture.

Lysozyme is an enzyme found in egg white, tears, and other secretions. It is responsible for breaking down the polysaccharide walls of many kinds of bacteria and thus it provides some protection against infection. The activity of enzymes is strongly affected by changes in pH and temperature. Each enzyme works best at a certain pH and temperature, its activity decreasing at values above and below that point. This is not surprising considering the importance of tertiary structure (i.e. shape) in enzyme function and non-covalent forces, e.g., ionic interactions and hydrogen bonds, in determining that shape. Since lysozyme is a natural form of protection from gram-positive pathogens like *Bacillus* and *Streptococcus* a deficiency due to infant formula feeding can lead to increased

incidence of disease. The effects of lysozyme under white fluorescent tube, results indicated that every concentrations of lysozyme induced best formation of PLB, shoot and root with modified MS media. Comparatively the PLBs which were dipping 30 minutes into lysozyme aqueous solution were best growth rate observed. Lysozyme is known to play a vital role in medical industry and the present study confirmed that lysozyme functions as a plant growth regulator in *Cymbidium insigne*. Lysozyme can stimulate PLBs proliferation of *Cymbidium in vitro*. Relatively low concentrations (0.1 mg/l) of lysozyme in culture media enhanced the maximum formation of PLB, shoot and root of *Cymbidium* spp. But the mechanism how it works in orchid tissue culture is unknown.

The response of methyl jasmonate (Me-JA) or jasmonic acid (JA) in the culture media as an elicitor enhanced the anthocyanin production of *Tulipa gesneriana* L. and *Vaccinium pahalae* Skottsb. [SANIEWSKI & al. 1998; FANG & al. 1999]. Methyl jasmonates (Me-JA) has successfully used as an elicitor in other plant species for enhancing the production of secondary metabolites in the cell cultures [AOYAGI & al. 2001; KIM & al. 2004; THANH & al. 2005]. Elicitation has been shown to be the most efficient strategy that direct to the enhancement in anthocyanin production in plant cell cultures [ZHANG & FURUSAKI, 1999). In *Cymbidium* tissue culture, Me-JA at 1 μM stimulated protocorm-like body (PLB) formation (from shoots) and shoot formation in epiphytic *Cymbidium eburneum* and in terrestrial *Cymbidum kanran* Makino [SHIMASAKI & al. 2003] while it stimulated, when applied at 1 mg/l, PLB formation from half-moon PLBs and PLB TCLs in a hybrid *Cymbidium* [TEIXEIRA DA SILVA, 2012]. According to this study result suggested that PLBs of *C. insigne* dipping 30 minutes at 0.1 mg/l Me-JA aqueous solution and cultured (after 30 minutes) on modified MS media, induced maximum formation of PLBs, shoot and root. When PLBs were dipping 60 minutes at different concentrations of Me-JA aqueous solution, 0.1 mg/l Me-JA induced maximum formation of PLB and shoot but root formation observed which PLBs were dipping at 1 mg/l Me-JA aqueous solution. Low concentrations of Me-JA (0.1 mg/l) induced best formation (PLBs, shoot and root) and 100 mg/l Me-JA aqueous solution had no shoot and root formation observed within culture period.

Conclusions

As biological control becomes more prevalent, useful, and important in horticultural crop production, targeted use of jasmonate-induced defenses may provide valuable augmentation of integrated pest management strategies. As reported by MIZUKAMI & al. (1993), jasmonic acid and its derivatives were involved in a part of the signal transduction pathway that induced particular enzymes catalyzing biochemical reactions for the synthesis of secondary metabolites and lysozyme is considered a “natural” antibiotic [GLYNN, 1968]. It is an important factor of innate immunity and a unique enzybiotic in that exerts not only antibacterial activity but also antiviral, anti-inflammatory, anticancer and immunomodulatory activities [SAVA, 1996; HELAL & al. 2012]. The results from this study indicate that Me-JA and lysozyme elicitation strategy was safe and useful to improve the PLBs culture of *Cymbidium insigne in vitro*.

References

AOYAGI H., KOBAYASHI Y., YAMADA K., YOKOYAMA M., KUSAKARI K. & TANAKA H. 2001. Efficient production of saikosaponins in *Bupleurum falcatum* root fragments combined with signal transducers. *Appl. Microbiol. Biotechnol.* **57**: 482-488.

DORNENBURG H. 2004. Evaluation of immobilization effects on metabolic activities and productivity in plant cell processes. *Process Biochem.* **39**(11): 1369-1375.

DU PUY D. & CRIBB P. 2007. *The Genus Cymbidium*. Richmond, UK: Kew Publishing, pp. viii-ix.

FANG Y., SMITH M. A. L. & PEPIN M. F. 1999. Effects of exogenous methyl jasmonate in elicited anthocyanin producing cell cultures of ohelo (*Vaccinium pahalae*). *In vitro Cell Dev. Biol. Plant.* **35**: 106-113.

GLYNN A. 1968. Lysozyme: Antigen, enzyme and antibacterial agent. *Sci Basis Med Annu Rev.* pp. 31-52.

HELAL R., BADER G. & MELZIG M. F. 2012. Stimulation of lysozyme release by selected microbial preparations. *Pharmazie.* **67**(6): 564-566.

KIM Y. S., HAHN E. J., MURTHY H. N. & PAEK K. Y. 2004. Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate *Biotechnol. Lett.* **26**: 1619-1622.

KOO A. J. K. & HOWE G. A. 2009. The wound hormone jasmonate. *Phytochemistry.* **70**: 1571-1580.

MEYER R., RAUTENBACH G. F. & DUBERY I. A. 2003. Identification and quantification of methyl jasmonate in leaf volatiles of Arabidopsis thaliana using solid-phase microextraction in combination with gas chromatography and mass spectrometry. *Phytochem. Anal.* **14**: 155-159.

MIZUKAMI H., TABIRA Y. & ELLIS B. E. 1993. Methyl jasmonate-induced rosmarinic acid biosynthesis in *Lithospermum erhythorhizon* cell supension cultures. *Plant Cell Rep.* **12**: 706- 709.

SANIEWSKI. M., MISZCZAK A., KAWA-MISCZAK L., WEGRZYNOWICZ-LESIAK E., MIYAMOTO K. & UEDA J. 1998. Effects of methyl jasmonate on anthocyanin accumulation, ethylene production, and CO_2 evolution in uncooled and cooled tulip bulbs. *J. Plant. Growth Regul.* **17**: 33-37.

SAVA G. 1996. Pharmacological aspects and therapeutic applications of lysozymes. *Experientia.* **75**: 433-449.

SEE K. S., BHATT A. & KENG C. L. 2011. Effect of sucrose and methyl jasmonate on biomass and anthocyanin production in cell suspension culture of *Melastoma malabathricum* (Melastomaceae). *Rev. biol. Trop.* **59**(2): 597-606.

SHIMASAKI K. & UEMOTO S. 1990. Micropropagation of a terrestrial *Cymbidium* species using rhizomes developed from seeds and pseudobulbs. *Plant Cell Tiss. Org. Cult.* **22**: 237-244.

SHIMASAKI K., SHIRAGA T. & FUKUMOTO Y. 2003. Effect of methyl jasmonate on organogenesis in shoot cultures of epiphytic and terrestrial *Cymbidium* species. *Env. Control Biol.* **41**(2): 179-182.

TEIXEIRA DA SILVA J. A. 2012. Impact of Methyl jasmonate on PLB formation of hybrid *Cymbidium* (Orchidaceae). *J. Plant Develop.* **19**: 47-52.

TEIXEIRA DA SILVA J. A. 2012a. Jasmonic acid, but not salicylic acid, improves PLB formation of hybrid *Cymbidium*. *Plant Tissue Cult. & Biotech.* **22**(2): 187-192.

THANH N. T., MURTHY H. N., YU K. W., HAHN E. J. & PAEK K. Y. 2005. Methyl jasmonate elicitation enhanced synthesis of ginsenoside by cell suspension cultures of *Panax ginseng* in 5-l balloon type bubble bioreactors. *Appl. Microbiol. Biotechnol.* **67**: 197-201.

YUN-SOO K., EUN-JOO H., HOSAKATTE N. M. & KEE-YOEUP P. 2004. Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate. *Biotechno. Lett.* **26**: 1619-1622.

ZHANG W. & FURUSAKI S. 1999. Production of anthocyanins by plant cell cultures. *Biotechnol. Bioproc. Eng.* **4**: 231-252.

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