

## IMPACT OF METHYL JASMONATE ON PLB FORMATION OF HYBRID *CYMBIDIUM* (Orchidaceae)

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**Abstract:** When methyl jasmonate (MeJA) was added at 1 mg/l, it could stimulate the development of protocorm-like bodies (PLBs) or PLB thin cell layers of hybrid *Cymbidium* Twilight Moon 'Day Light', when added to Teixeira *Cymbidium* (TC) medium without plant growth regulators. This is a simple means to mass produce PLBs for commercial purposes.

**Key words:** MeJA, PGR, protocorm-like body or PLB, Teixeira *Cymbidium* (TC) medium, thin cell layer or TCL

### Introduction

Ethylene and methyl jasmonate (MeJA) pathways are often interlinked and related to plant development [SANIEWSKI & al. 2002] and defence [ALMAGRO & al. 2009]. MeJA stimulated the genes involved in lignin biosynthesis [YAQOOB & al. 2012]. MeJA at 1  $\mu$ M stimulated protocorm-like body (PLB) formation (from shoots) and shoot formation in epiphytic *Cymbidium eburneum* Lindley and in terrestrial *Cymbidium kanran* Makino [SHIMASAKI & al. 2003] and this possibility served as the basis for assessing in this study whether the same would be true, and possible, for hybrid *Cymbidium*. Limited other studies on the effects of MeJA on plant growth *in vitro* exist although MeJA was shown to enhance flavonolignan production 300% in *Silybum marianum* liquid root cultures [ELWEKEEL & al. 2012], typical of jasmonates, which, in their capacity as a stress hormone, stimulate secondary metabolite production in a wide range of plant cell cultures [KOO & HOWE, 2009]. *Lilium* bulb formation was enhanced *in vitro* in the presence of MeJA [JASIK & DE KLERK, 2006] as was potato tuberization *in vitro* [SARKAR & al. 2006].

*In vitro* protocols for the induction and development of protocorm-like bodies (PLBs) of hybrid *Cymbidium* are well established. Despite this, no study exists yet on the use of and effect of MeJA *in vitro* on hybrid *Cymbidium*. Thus, using a newly developed ideal *Cymbidium* PLB regeneration medium, termed Teixeira *Cymbidium* (TC) medium [TEIXEIRA DA SILVA, 2012], the effect of MeJA on PLB formation was assessed with the expectation of increasing PLB formation.

### Materials and methods

#### *Chemicals and reagents*

All chemicals and reagents were of the highest analytical grade available and were purchased from either Sigma-Aldrich (St. Louis, USA), Wako (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan), unless specified otherwise.

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### *Plant material and culture conditions*

PLBs (either half-moon PLBs or PLB TCLs: see TEIXEIRA DA SILVA, 2013) of hybrid *Cymbidium* Twilight Moon 'Day Light' (Bio-U, Japan) originally developed spontaneously from shoot-tip culture on Vacin and Went (VW, 1949) agar medium without PGRs, were induced and subcultured (PLB induction and proliferation medium or VWPLB) every two months on TC medium [TEIXEIRA DA SILVA, 2012], which contains unique levels of macro- and micronutrients, and was supplemented with two plant growth regulators, or PGRs (0.1 mg/l  $\alpha$ -naphthaleneacetic acid (NAA) and 0.1 mg/l kinetin (Kin)), 2 g/l tryptone and 20 g/l sucrose, and solidified with 8 g/l Bacto agar (Difco Labs., USA), according to procedures and advice outlined by TEIXEIRA DA SILVA & al. (2005) and TEIXEIRA DA SILVA & TANAKA (2006).

All media were adjusted to pH 5.3 with 1 N NaOH or HCL prior to autoclaving at 100 KPa for 17 min. Cultures were kept on 40 ml medium in 100-ml Erlenmeyer flasks, double-capped with aluminium foil, at 25 °C, under a 16-h photoperiod with a light intensity of 45  $\mu\text{mol}/\text{m}^2\text{s}$  provided by plant growth fluorescent lamps (Homo Lux, Matsushita Electric Industrial Co., Japan). Longitudinally bisected PLB (3-4 mm in diameter) segments, 10 per flask, were used as explants for PLB induction and proliferation and for all experiments. Culture conditions and media followed the recommendations previously established for medium formulation [TEIXEIRA DA SILVA & al. 2005], biotic [TEIXEIRA DA SILVA & al. 2006b] and abiotic factors [TEIXEIRA DA SILVA & al. 2006a] for PLB induction, formation and proliferation.

### *Effect of ethylene inhibitors on PLB formation*

Half-moon PLBs and PLB thin cell layers (TCLs) were cultured on PGR-free TC or PGR-containing TC medium in the presence of 1, 2, 4 or 8 mg/l MeJA. The control contained no PGRs and no MeJA. Solutions were made fresh and were filtered prior to the addition to TC medium.

### *Morphological parameters assessed*

The number of PLBs formed per PLB segment or PLB TCL was measured after 45 days in culture since PLBs induce shoots spontaneously when left in culture on the same medium for more than 45 days. In this study, shoots are not desired, only PLBs since PLBs are the ideal propagative medium and somatic embryos for use in other studies. PLBs form on the surface of PLBs only [TEIXEIRA DA SILVA & TANAKA, 2006].

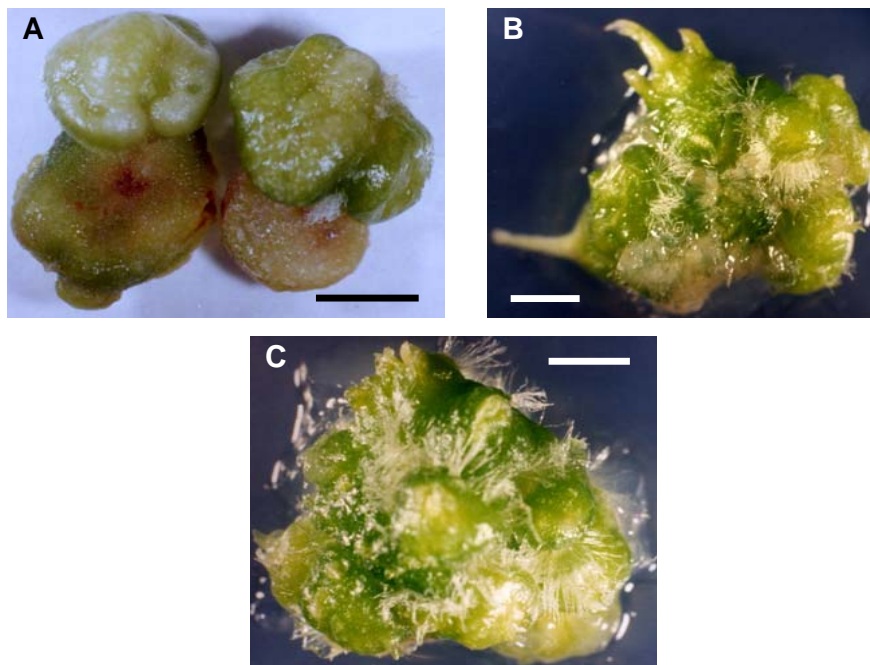
### *Statistical analyses*

Experiments were organized according to a randomized complete block design (RCBD) with three blocks of 10 replicates per treatment (i.e., each medium). All experiments were repeated in triplicate ( $n = 90$ , total sample size per treatment). Data was subjected to analysis of variance (ANOVA) with mean separation by Duncan's new multiple range test (DNMRT) using SAS® vers. 6.12 (SAS Institute, Cary, NC, USA). Significant differences between means were assumed at  $P \leq 0.05$ .

## **Results and discussions**

The addition of 1 mg/l of MeJA to PGR-free TC medium improved PLB formation significantly relative to PGR-free control TC, although PLB formation decreased as MeJA level increased (Tab. 1). MeJA is thus a simple and cost-effective way to improve PLB production for micropropagation purposes, serving as an alternative to PGRs (Fig. 1).

The response of MeJA, as a subset of jasmonates, is usually in response to a biotic or abiotic stress and is thus generally associated with growth inhibition (for example *Medicago sativa* somatic embryogenesis; RUDUŠ & al. 2006) rather than growth promotion, although this can be beneficial for secondary metabolite production *in vitro* where it serves as an elicitor [KOO & HOWE, 2009]. Consequently, MeJA is not frequently observed as a useful PGR and thus the number of studies remains limited. It is however, frequently used as a postharvest treatment to suppress fungal infections in fresh produce [e.g., GONZALEZ-AGUILAR & al. 2003]. Transgenic soybean plants with enhanced MeJA expression showed strongly different morphology in leaves and roots [XUE & ZHANG, 2007] while somatic embryogenesis-related genes (*Lea*) were activated in *Nicotiana plumbaginifolia* [REINBOTHE & al. 1994]. TCLs were also used to enhance adventitious root production in tobacco after exposure to MeJA [FATTORINI & al. 2009] although, interestingly, it disrupted TCL-derived shoot formation, also in tobacco, by over-inducing mitotic activity and cell expansion [CAPITANI & al. 2005]. MeJA also reduced microrhizome biomass in turmeric cultures [COUSINS & ADELBERG, 2008]. PLBs and somatic embryos are synonymous in orchids [TEIXEIRA DA SILVA & TANAKA, 2006], and thus this protocol, using half-moon PLBs or PLB TCLs, represents a means of producing PLBs where a bioreactor is not available. Simple techniques that allow for increasing PLB production are central to orchid biotechnology [HOSSAIN & al. 2013].



**Fig. 1.** (A) Thin cell layer forming PLBs 45 days after culture on Teixeira *Cymbidium* medium (TCPLB) with PGRs (0.1 mg/l  $\alpha$ -naphthaleneacetic acid + 0.1 mg/l kinetin). (B) PLB induction from half-moon PLBs on TCPLB. (C) PLB formation on TCPLB without plant growth regulators but supplemented with 2.0 mg/l methyl jasmonate. Bars = 1 mm (A), 5 mm (B, C).

**Tab. 1.** Effect of methyl jasmonate on new/*neo*-PLB formation from half-PLB or PLB TCL culture of hybrid *Cymbidium* Twilight Moon ‘Day Light’.

Medium composition	MeJA concentration (mg/l)	Percentage of explants forming <i>neo</i> -PLBs (%)	Number of PLBs per explant	Fresh weight (mg) of PLB explant + <i>neo</i> -PLBs
Half-moon PLBs on: TC (control) PGR-free TC		100 a	8.3 b	526 a
		81 ab	6.1 bc	431 ab
PLB TCLs on: TC (control) PGR-free TC		100 a	1.2 e	321 b
		76 ab	0.4 f	81 cd
Half-moon PLBs on: PGR-free TC + MeJA	1	100 a	9.6 a	518 a
	2	100 a	4.8 c	341 b
	4	67 b	2.6 d	186 c
	8	21 c	1.1 e	98 c
PLB TCLs on: PGR-free TC + MeJA	1	100 a	3.6 cd	324 b
	2	100 a	1.4 e	303 b
	4	78 b	0.8 ef	118 bc
	8	46 bc	0.3 f	68 d
<p>Mean values followed by the same letter in the same column are not significantly different based on DMRT (<math>P = 0.05</math>). See text for media constituents. n = 90 (9 Petri dishes × 10 for each treatment).</p> <p>MeJA, methyl jasmonate; PGR, plant growth regulator; PLB, protocorm-like body; TC, Teixeira <i>Cymbidium</i> medium [TEIXEIRA DA SILVA, 2012], includes 0.1 mg/l <math>\alpha</math>-naphthaleneacetic acid and 0.1 mg/l kinetin, 2 g/l tryptone and 20 g/l sucrose (see reference for modified micro- and macro-nutrients); TCL, thin cell layer</p>				

### Conclusions

Half-moon PLB explants or PLB TCLs can be used to derive new or *neo*-PLBs in hybrid *Cymbidium*. MeJA has shown to alter growth and developmental properties in plants, including other *Cymbidium* spp., and in this study, at least in hybrid *Cymbidium*, it could enhance PLB production relative to ideal PGR-containing medium. Although TCLs overall produce fewer *neo*-PLBs/PLB explant than half-moon PLBs, after conversion with the Plant Growth Correction Factor (TEIXEIRA DA SILVA & DOBRÁNSZKI, 2011), and based on surface area of explants, the potential number of PLBs in fact exceeds the recorded number. MeJA thus serves as an alternative means to derive *neo*-PLBs.

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### Abbreviations:

MeJA – methyl jasmonate; NAA –  $\alpha$ -naphthaleneacetic acid; PLB – protocorm-like body; PGR – plant growth regulator; TDZ – thidiazuron (N-phenyl-N-1,2,3-thidiazuron-5'-ylurea); VW – Vacin and Went

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