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OPINION PAPER: SHOULD THE TERM PROTOCORM-LIKE BODY BE USED EXCLUSIVELY FOR ORCHIDS?

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The term protocorm-like body (PLB) is traditionally used to describe an organ that develops in orchid *in vitro* culture that resembles, in morphology (structure) and function, an enlarged seed-derived zygotic embryo, the protocorm [ARDITTI, 1979]. The term protocorm was coined by Melchior Treub for seedlings of club mosses while studying the sporophyte developmental stages of cormophytes [TREUB, 1890]. Noel Bernard then directly applied Treub's concept of protocorm (for structures originating from seed) to the Orchidaceae [BERNARD, 1909]. A protocorm is "the tuber-like swollen part of an orchid seed, which appears during the early stage of germination" [XIONG, 2009] or, according to the online Merriam-Webster dictionary, "a tuber-shaped body with rhizoids that is produced by the young seedlings of various orchids and some other plants having associated mycorrhizal fungi". MOREL (1960) may very well have been the first orchidologist to describe a PLB as "an uncertain term that means a structure formed *in vitro* that looks similar to a protocorm" [YAM & ARDITTI, 2009]. However, a PLB is a *de facto* somatic embryo – an embryo that is derived from a somatic cell – in orchids [TEIXEIRA DA SILVA & TANAKA, 2006; LEE & al. 2013, and references therein]. This suggests that a PLB is an organ specific to the Orchidaceae. Indeed, the first public call for the strict use of the term PLB in orchids dates back to *Phalaenopsis* research [ISHII & al. 1998] and is a term that has been used exclusively for orchids in tissue culture in thousands of scientific papers. The plant literature thus suggests that the terms protocorm and PLB could be applied primarily to orchids, but could also be used more widely.

However, the term "PLB" has been used to describe, for at least 11 non-orchidaceous plants, round, globular structures that resemble PLBs in other crops, primarily ornamental plants: *Anthurium andraeanum* (Araceae) [GANTAIT & al. 2012, based on YU & al. 2009], Brodiaea (*Dichelostemma congestum*, *Triteleia laxa*, *T. ixioides*, *T. hyacintina*; ILAN & al. 1995), *Colocasia esculenta* (Araceae) [ABO EL-NIL & ZETTLER, 1976; NYMAN & al. 1983; NYMAN & ARDITTI, 1988; NYMAN & al. 1989; SABAPATHY & NAIR, 1992], *Heliconia psittacorum* (Heliconiaceae) [NATHAN & al. 1993; GOH & al. 1995; KUMAR & al. 1996], *Hippeastrum hybridum* (Amaryllidaceae) [HUANG & al. 1990], *Lilium longiflorum* (Liliaceae) [NHUT & al. 2001, 2002], *Musa* [VENKATACHALAM & al. 2006], *Philodendron micans* (Araceae) [XIONG, 2009], *Pinellia ternata* [WANG & al. 2009; LIU & al. 2010a, 2010b], *Rosa* [TIAN & al. 2008; LIU & al. 2014], and *Syngonium podophyllum* (Araceae) [CUI & al. 2008].

In fact, closer analysis of the literature reveals the following (when searching for the terms protocorm-like body or PLB): 4130 hits on Google Scholar (mostly orchids); 213

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hits on Elsevier's Sciencedirect.com (7 non-orchids); 180 hits on Springer Science + Business Medium's SpringerLink (9 non-orchids); 13 hits on Taylor and Francis, Wiley-Blackwell and deGruyter Online platforms (1 non-orchid). Thus, it is estimated that from the total main-stream literature, that approximately 95% of the plant science literature uses the term PLB for orchids.

The CUI & al. (2008) paper shows light-microscopic images of round structures which, using histological sectioning, appear to be somatic embryos. How then did the authors extrapolate to conclude that such structures were PLBs? The authors appear to rely on the literature to support their claims. For example, "Protocorm-like bodies are composed of many meristematic centers that are able to differentiate into shoots and roots [DA SILVA & al. 2000]." However, examination of the original DA SILVA & al. (2000) paper reveals that the authors did not use the term PLB at all, instead, the authors were describing the development of plantlets from pro-embryoids following anther culture. The CUI & al. (2008) paper thus incorrectly describes the literature and inadvertently introduces a significant error that affects the subsequent use of the term PLB in non-orchid genera. Is then the use of the term "PLB" for plants that are not orchids a misnomer (i.e., "a term or meaning that suggests a meaning that is known to be wrong" (Wikipedia 2014)? NATHAN & al. (1993) first used the term to describe PLBs in *Heliconia psittacorum* what were clearly somatic embryos, but no botanical explanation as to why the term PLB was used, was provided. The term PLB is then promulgated by the same authors in subsequent papers [GOH & al. 1995; KUMAR & al. 1996]. GANTAIT & al. (2012), despite accurately describing the exclusive use thus far in the plant science literature for the Orchidaceae, then suddenly employ it for *Anthurium*, the only rationale being that term had been used previously by YU & al. (2009). No other botanically-based rationale is provided. In fact, an examination of the YU & al. (2009) study reveals that the rationale for the use of the term PLB is incorrect, flawed and botanically unfounded: "Induction of protocorm-like bodies (PLBs) is a popular method to *Phalaenopsis* [ROY & al. 2007]. To our knowledge, there is no report of regeneration from protocorm-like bodies (PLBs) formation of anthuriums." Reference to the ROY & al. (2007) study is in fact also incorrect since that study is on *Dendrobium*, and not *Phalaenopsis*, calling into question thus the scientific and botanical accuracy of the NATHAN & al. (1993), CUI & al. (2008), YU & al. (2009) and GANTAIT & al. (2012) studies, specifically the use of the term PLB.

HUANG & al. (1990) decided to refer to the production of bulblets, a well-established term for the small bulblets that form from single or twin scales in the Liliaceae and Amaryllidaceae, PLBs, without any logical reason or explanation. It is unclear if any literature pertaining to bulblet formation in *Hippeastrum* adopted this term, but further scrutiny of the *Hippeastrum* literature is merited. In the Liliaceae, specifically *Lilium longiflorum*, NHUT & al. (2001) first introduce the term PLB to describe what is interchangeably referred to as pseudo-bulblets or somatic embryos, but then, without any clear explanation, adopted as PLBs. The potentially incorrect term is then carried forward to an ensuing study [NHUT & al. 2002]. A similar error to these studies was made by ILAN & al. (1995) in half a dozen members of the Brodiaea, in which cormlets were referred to as PLBs, without any botanical explanation or rational to substitute an already well-established term, or histological proof. NYMAN & ARDITTI (1988) observed several structures all forming simultaneously from the same explants, but refer to the round ones as PLBs. However, in their study, the nomenclature is inconsistent, sometimes referred to as

protocorm-like bodies, and sometimes as protocorm-like structures, weakening thus the rationale for the use of the term PLB for taro, a tuberous crop.

XIONG (2009) stated in the abstract "Histological analysis suggested that the globular structures were protocorm-like bodies (PLBs), a novel pathway for plant regeneration." Xiong then uses the term PLB for *Philodendron*, basing his defense of the use of this term and his rationale on the fact that "PLBs have been identified in a wide range of other plant genera." However, this characterization of the literature is incorrect and thus misleading. In fact, what Xiong observed was simply the use of the term PLB in the literature, but not necessarily the correct assignment of the term. However, the developmental evidence provided by Xiong in Fig. 3-1 A and B of the thesis seems to indicate that these round structures are simply undeveloped shoot initials while Fig. 3-2 suggests that indeed these structures may be somatic embryos or somatic-embryo-like structures. If so, then why did Xiong simply not refer to them as somatic embryos rather than PLBs?

TIAN & al. (2008) first induced callus and new rhizoids from *Rosa* spp. (*R. canina* L., *R. multiflora* var. *cathayensis* Rehd. & Wils., and *R. multiflora* f. *carnea* Thory.) rhizoids, then, after transfer to a "PLB-formation medium", which contained thidiazuron, then induced what they claimed were PLBs. However, examination of the structures they termed PLBs reveals structures that were anything except what is typically observed in orchids, thus begging the question: why did the authors select this term rather than creating a new term? The authors offer absolutely no botanical explanation for their choice of term, and the structures they observe appear to be either undeveloped shoot initials, or hyperhydric shoots, albeit with a roundish structure. Ironically, the biggest clue that in fact these are not PLBs, which develop only from epidermal layers, comes precisely from the evidence which the authors claim proves that these are PLBs: histological sections. Their Fig. 3d indicates a PLB forming in the center of parenchymatous cells (described in their figure legend as a meristematic center). Thus, this is clearly not a PLB, but some other structure. The same error exists in a subsequent paper by the same group that further tries to fortify the use of the term PLB for *R. canina* [LIU & al. 2014]. In that paper, despite an impressive display of histological analyses, essentially the authors observe "green ellipsoidal bodies", or "deep-green globular bodies" at the tips of rhizoids, i.e., inconsistent nomenclature, but then chose to use the term PLB to describe these structures, without indicating why botanically they should be named as such. Their histological analysis shows a mélange of shoots initials and "PLBs", which histologically are indistinct, once again reinforcing the question: why were these green round structures termed PLBs?

LIU & al. (2010a) identify the formation of round, green structures on *Pinellia ternata* leaf, petiole and tuber explants in the presence ofα- naphthalene acetic acid (NAA) and 6-benzyladenine (BA), but no histological proof is provided. The structure, claimed to be a PLB, is then used as the basis of the explants in a subsequent study [LIU & al. 2010b]. The use of the term PLB is extremely problematic since the same authors identified the exact same structures as micro-tubers in the same plant, using the same explants and the same plant growth regulators, BA and NAA [WANG & al. 2009]. In that study, the botanical name of the plant was also incorrectly spelt.

Thus, the initial use of the term PLB for non-orchids is, in my opinion, flawed, and unexplained, at least in botanical terms. Moreover, absolutely no rationale has been provided by these 11 non-Orchidaceous studies and very rarely (only 2-3 recent studies) has

cytological evidence been provided. Consequently, by association, all references that lend support to these potentially flawed studies would themselves be flawed, by association.

The ability of scientists to apparently easily introduce, even following "peer review", neologisms easily into the scientific literature without a sound or accurate scientific basis is one of the unintended (and unfortunate) consequences of the lack of defined standards regarding some terminology used in plant tissue culture and plant development, and also indicates one of the weaknesses of traditional peer review [TEIXEIRA DA SILVA & DOBRÁNSZKI, 2015], even in established plant science journals. The real risk that the plant science community faces is that any round, somatic embryo-like or PLB-like structure that visually resembles a PLB will be termed a PLB in any plant species when in fact those plants do not form a protocorm from their seed. Thus, the botanically restricted term will lose its importance and impact, and exclusivity, for the orchids.

For example, it is difficult to imagine PLB formation in tobacco, potato, or *Arabidopsis thaliana*. However, by creating an exceptional precedent, these 11 studies now provide a spring-board for the unbased and expanded use of this term to non-Orchidaceous plants, which I feel is problematic. Undoubtedly, the "originality" factor would certainly score the authors of such papers a publication since reviewers who would be reviewing such papers would feel that a "new botanical structure" had been discovered in that plant when in fact no such botanical basis exists. However, botanically-speaking, would the use of this term for non-orchidaceous plants be accurate, or correct?

This paper then calls on the standardized use of the term protocorm-like body, or PLB, to describe somatic embryogenesis in orchids (all genera) and that structures that resemble PLBs in any other plant family be referred to more accurately as PLB-like bodies (i.e., protocorm-like body-like bodies). No doubt that this will be a topic of discussion until more irrefutable evidence, and solid logic, is provided that champions for the exclusive use of the term PLBs in the Orchidaceae. Moreover, provided that different levels of quality control exist in editor boards of plant science journals published by different publishers, and in a non-standard interpretation of the developmental aspect of orchids by so-called "peer reviewers", it will be difficult to ensure a literature-wide control of the use of suitable terminology. Studies like those by TIAN & al. (2008) and LIU & al. (2014) lend credence to the broader use of PLBs to non-Orchidaceous crops, while other studies that simply use the term PLB to describe a round, green structure without detailed histological or developmental analyses may very well be diluting, or even corrupting, the botanical literature.

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"I understand and agree with your point. To me, PLBs are exclusive to orchids, but technically, others might have an argument for expanding the use of the term. Just playing devil's advocate…" Wagner A. Vendrame

"Your question is a matter of opinion more than definition. If you want to be strict you are right. I would opt for a broader use." Joseph Arditti (Professor of Biology Emeritus, University of California – Irvine, USA)

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